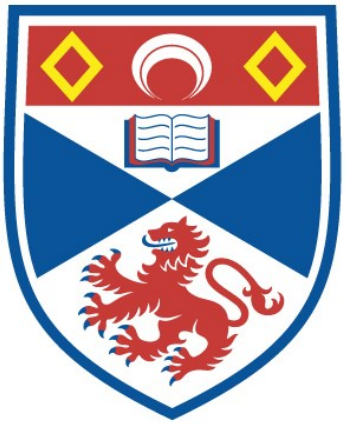
TEMPERATURE AND ITS EFFECTS ON SOME MARITIME PLANTS IN BRITAIN

M. Anne Palin

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1979

ABSTRACT

Temperature and its Effect on some Maritime Plants in Britain.

The physiological ecology of five coastal species has been examined with respect to temperature and its effect on survival and distribution. The aims of the study have been to establish whether any direct correlation exists between distribution and the responses of the plants to temperature at different stages in the life cycle. The species under consideration were the northern Ligusticum scoticum and Mertensia maritima and the southern Crithmum maritimum, Limonium binervosum and Glaucium flavum.

Highest germination percentages for each species were found at temperatures close to those associated with the season favourable for germination in the natural habitat. Northern species had higher temperature requirements than the southern, corresponding to spring/summer and autumn or spring germination respectively.

Root respiration, measured as oxygen uptake, was found to be twice as great in the northern Ligusticum and Mertensia as in the southern Crithmum and Limonium over a range of experimental temperatures. This varied to some extent with time and temperature of pretreatment. The single experiment on the southern Glaucium showed rates similar to those of the northern species. Arrhenius plots of respiration data for the northern species showed a break in gradient at the upper end of the experimental temperature range which correlated well with apparently limiting July mean temperatures from the distribution maps. The southern Crithmum showed a break at lower temperature range close to the limiting January mean temperature. The response of Limonium to experimental temperature depended on the pretreatment; upper range breaks were shown after low pretreatment temperatures, and lower range breaks after higher pretreatment temperatures. The single experiment on Glaucium gave a straight line Arrhenius plot.

Carbohydrate analyses of the same pretreated plants yielded additional information relevant to the survival and thus to the distribution in relation

to temperature. The southern Crithmum had the highest starch content at all temperatures while the northern Ligusticum and Mertensia had less. Ratios of soluble sugar to starch were greatest in the northern species, possibly reflecting displacement of the equilibrium from starch to soluble sugar at lower temperatures.

Overall a connection has been demonstrated between the direct effects of temperature on the plants and the limitation of distribution by temperature. This is clearest for the two northern species, Ligusticum scoticum and Mertensia maritima, less definite for the southern Crithmum maritimum, and only suggested for Limonium binervosum with its apparently less simple temperature responses. Glaucium flavum appears anomalous and requires further study.

Temperature and its Effect on some Maritime Plants in Britain.

A thesis presented for the degree of Ph.D.
at the
University of St. Andrews
1979

by

M. Anne Palin B.A. (Oxon)

Declaration

I hereby declare that this thesis has been composed by myself, and that it is a record of work done by myself. None of the work has been accepted in any previous application for a degree. Any other sources of information have been individually acknowledged.

Signed:

M.Anne Palin

I, Margaret Anne Palin, was admitted as a research student of the University of St. Andrews in October 1975 in accordance with Ordinance General No. 12, and the resolution of the University Court, 1967, No. 1. The thesis was completed in March 1979.

Certificate

I hereby certify that Margaret Anne Palin has been engaged upon research from October 1975 onwards to prepare the accompanying thesis for the degree of Doctor of Philosophy, and has completed the required number of terms.

Signed:

R.M.M. Crawford

St. Andrews
March 1979

Acknowledgements

I would like to thank Professors J.A. Macdonald and D.H.N. Spence for making available facilities in the Botany Department, St. Andrews, and Professor R.M.M.Crawford for all his help and advice as my research supervisor.

I am grateful to Mr. R. Mitchell of the St. Andrews Botanic Garden, for obtaining plants and seed, and to Mr. C.Preston (of Cambridge University), Miss E.Bullard (of Orkney), Dr. J.E.Palin and Dr. and Mrs.D.E.Palin for conservation-conscious collection of plants from the wild.

I wish to acknowledge, with thanks, permission from the Biological Records Centre, Monks Wood, to reproduce the distribution maps used in this thesis.

The last year of the work was funded by a University of St. Andrews Research Scholarship for which I am most grateful.

CONTENTS

	Page
CHAPTER 1 : INTRODUCTION	1
CHAPTER 2 : CORRELATIONS OF COASTAL SPECIES WITH TEMPERATURE DATA	6
INTRODUCTION	6
NORTHERN SPECIES	9
A. General Considerations	9
Table 2.1	10
B. Detailed Consideration of Typical Species	12
a. Ligusticum scoticum	12
b. Mertensia maritima	13
Photos 2.1 and 2.2	15
Figures 2.3 and 2.4	16
SOUTHERN SPECIES	18
A. General Considerations	18
Table 2.2	20
B. Detailed Consideration of Typical Species	26
1. Winter Temperature Limitation	26
a. Crithmum maritimum	26
Photo 2.3	27
b. Spergularia rupicola	28
2. Summer Temperature Limitation	28
a. Halimione portulacoides	28
b. Limonium vulgare	29
Figures 2.5 to 2.8	30
3. Winter and Summer Limitations	34
a. Limonium binervosum	34
b. Glaucium flavum	34
c. Inula crithmoides	35

	Page
Figures 2.9 to 2.11	37
CONCLUSION	40
Figures 2.1 and 2.2 (fold out)	41
CHAPTER 3 : THE EFFECT OF TEMPERATURE ON SEED GERMINATION OF COASTAL SPECIES	43
INTRODUCTION	43
Table 3.1	46
MATERIALS AND METHODS	47
1. Constant Temperature	48
2. Alternating Temperatures	48
3. Temperature and Light Interaction	48
RESULTS AND DISCUSSION	50
1. Constant Temperatures	50
Tables 3.2	54
Figure 3.1	57
Table 3.3	58
Figure 3.2	59
Tables 3.4 to 3.6	60
Figure 3.3	63
2. Alternating Temperatures	64
3. Temperature and Light Interaction	66
Table 3.7	68
Figure 3.4	70
Table 3.8	71
CONCLUSIONS	72

Page

CHAPTER 4 : THE EFFECT OF TEMPERATURE ON ROOT RESPIRATION 74

INTRODUCTION 74

Table 4.1 77

MATERIALS AND METHODS 78

Gilson Respirometer 82

RESULTS 83

A. Basic Respiration Data 83

Figure 4.1 87

Tables 4.2 to 4.9 88

Figures 4.2 to 4.5 96

B. Transformed Respiration Data 100

Tables 4.10 to 4.15 106

Figures 4.6 to 4.14 112

DISCUSSION AND CONCLUSIONS 121

CHAPTER 5 : EFFECT OF TEMPERATURE ON WHOLE PLANTS 130

INTRODUCTION 130

MATERIALS AND METHODS 130

1. Incidental observations on the effect of pretreatment at a range of temperatures 130

2. Whole plant reaction of Ligusticum scoticum to pretreatment at a range of temperatures 130

RESULTS 132

1. Incidental observations on the effect of pretreatment at a range of temperatures 132

2. Whole plant reaction of Ligusticum scoticum to pretreatment at a range of temperatures 133

Tables 5.1 to 5.4 134

Photo 5.1 138

DISCUSSION AND CONCLUSIONS 139

Page

CHAPTER 6 : EFFECT OF TEMPERATURE ON CARBOHYDRATE CONTENT OF ROOTS 141

INTRODUCTION 141

METHODS 143

1. Starch estimation 143

2. Total soluble sugar estimation 144

Anthrone Reagent 144

3. Characterisation and determination of sugars by gas liquid chromatography 145

RESULTS 146

1. Starch 146

Tables 6.1 to 6.3 147

2. Total soluble sugars 149

3. Starch and sugars considered together 149

Tables 6.4 to 6.9 152

4. Sugar characterisation and determination 156

Table 6.9 157

DISCUSSION AND CONCLUSIONS 158

CHAPTER 7 : SUMMARY AND CONCLUSIONS 160

APPENDIX 1 171

REFERENCES 172

ADDENDA 176

CHAPTER 1 - INTRODUCTION

There have been many different approaches made to the study of distribution of plants in relation to temperature. For example, from observations on Scandinavian alpine and arctic species, Dahl (1951) pointed out correlations between their southern limits and isotherms for maximum summer temperatures. Similarly, Seddon (1971) pointed out a number of close correlations between summer and winter mean isotherms and plant distributions in Britain. These conclusions were drawn from observation of relevant distribution maps and corresponding overlays (ed. Perring and Walters, 1976). These studies demonstrate a connection between distribution and temperature, but the relationship is not explained except as a probable function of length of growing season in day degrees.

The limitations of temperature on the distribution of a plant species may act at any stage in the life cycle of the plant. The constraint can be on seed germination, seedling establishment, or on the mature plant, and may occur in either summer or winter. If temperature acts directly on the plant, then the effects will possibly be noticeable in the short term, over say, two or three years. If temperature is affecting such processes as flowering and seed set, then the effect may only be noticeable over a longer period of, say, 10 to 15 years. The actual time over which the temperature constraint is apparent will depend on the longevity of the plant and on variations in climate from year to year. Several of the above factors are considered in more detail below.

Various studies on seed germination have shown the many ways in which seed of different species responds to temperature. Optimum germination requirements vary widely and depend on a combination of the storage temperature and the germination temperature, and whether these are constant or fluctuating. Light may also interact with temperature and the resulting total germination characteristics are unique for each species or ecotype. Examples of work of this kind on a wide variety of species are to be found

in papers by Thompson (1973,1974a,1974b,1974c), Probert and Thompson (1976) and Simon et al (1976) in addition to the many examples cited by Mayer and Poljakoff-Mayber (1975).

Work on temperature sensitivity of seedlings has often been included with a study of mature plants. However, seedlings may be more sensitive to adverse temperature conditions than adult plants as stated by Okusanya (1976) for Crithmum maritimum L.,[1] and they may also be more sensitive to such non-temperature related pressures as grazing or trampling than adult plants.

The survival of any particular mature plant must depend ultimately on the maintenance of a positive carbohydrate balance, though this is in addition to its ability to survive such ecological pressures as shading, extreme oligotrophy or eutrophy, and competion from other species. The combination of these pressures with sub-optimal or limiting temperatures, whatever the temperature effect, will ultimately determine whether or not the plant will survive in any one place and will thus contribute to the determination of its overall distribution.

In Britain, Stewart and Bannister (1974) have shown that three species of Vaccinium have different responses to temperature which can be linked with their distributions in Britain. These three, V.myrtillus L., V.vitis-idaea L. and V.uliginosum L. show progressively more northern distributions, in that order, and measured dark respiration rates are highest in the most northerly species, V.uliginosum. This is in accordance with the observations of many authors, reviewed by Billings and Mooney (1968), that arctic and alpine species tend to have higher intrinsic rates of respiration than temperate or lowland species. Stewart and Bannister (1973) also studied the carbohydrate status throughout the year, particularly for V.uliginosum, and found a characteristic yearly cycle of rising and falling levels of carbohydrate. This cycle was easily correlated with seasonal

1 - Nomenclature throughout is from Clapham, Tutin and Warburg (1962)

changes in temperature and light intensity, and in conjunction with data in the 1974 paper, the different parts played by photosynthesis and respiration in winter were calculated.

Work in the U.S.A. has shown variations in carbohydrate status in relation to growth and respiration rate for various alpine plants from different altitudes and latitudes (Mooney and Billings, 1960,1961,1965; Mooney, 1963). Temperature was suggested as the limiting factor here, though it was only treated as a function of altitude in some instances.

More recently Higgins and Spomer (1976) have demonstrated that soil temperature is more important than air temperature in the effect it has on long term survival of plants. Here, the temperature of the roots was related directly to the respiration rate and survival potential of the plants.

Wager (1941) also studied respiration and carbon assimilation in relation to temperature for a range of arctic species and again confirmed that those of most northerly distribution tended to have high intrinsic rates of respiration especially when compared with species of temperate distribution. These data were also compared with those from other authors for a variety of species including crop plants.

Most of the work so far discussed has been concerned with wild plants of arctic or alpine origin from various parts of the world. The arctic species are limited in their distribution almost exclusively by latitude and related temperature, and they thus have a reasonably continuous habitat where there is land. Though the relationship between altitude and temperature is reasonably simple, the limitations to the study of distribution of alpine plants are greater than those for arctic ones because the disjunct habitat may prevent a species from occupying its full potential range.

In order to avoid at least some of these problems, the present study has been restricted to plants with coastal habitats so that discontinuity of habitat will not seriously limit distribution; even though the coast

has variety such as cliff, shingle, dune and salt-marsh habitats. In addition both the altitudinal range, and annual and diurnal temperature fluctuations of the coast are relatively limited when compared with inland sites.

Consequently it was decided to initiate the present investigation on temperature limitations on distribution of selected coastal species found in Britain. To facilitate the choice of suitable species for study, and to enable comparisons to be made, a list of coastal species with limited distributions in Britain was made from the 'Atlas of the British Flora' (ed. Perring and Walters, 1976) together with their habitats, distribution limits in Britain and the rest of the world, and corresponding apparently limiting isotherms in Britain. This information is given and considered further in Chapter 2.

Two of these apparently temperature limited coastal plants were chosen initially for study; one northern, Ligusticum scoticum L., Scots Lovage; and one southern, Crithmum maritimum L., Rock Samphire. Both are members of the Umbelliferae and are found in similar habitats on rocky shores and cliffs. These two species were treated the most thoroughly in the experimental work.

Subsequently a further northern species, Mertensia maritima(L) S.F.Gray, Oyster Plant, and a further southern species, Limonium binervosum (G.E.Sm) C.E. Salmon, Rock Sea-lavender, were added to the study for comparison but these were not treated so thoroughly. Some limited experiments were also carried out on Glaucium flavum Cranz, Yellow Horned-poppy, another southern plant. Material both as seed and mature plants was limiting to some extent for all five species, but in particular there were shortages of seed for Mertensia[1] and Limonium and of plants for Glaucium.

A brief ecological description of these five species is also given in Chapter 2 together with those for several other coastal species for

1 - The generic name only is used for brevity throughout except in cases where confusion might otherwise occur.

comparison. The distribution of each plant is compared with temperature data and any apparent correlations between the two are noted.

Some of the possible stages in the life cycle of the plants at which temperature may be restricting the distribution were then investigated. One such stage is seed germination, and Chapter 3 comprises an account of the effect of a range of experimental temperature regimes on this. All species were tested under constant temperature conditions, and some additional alternating day and night temperature experiments were carried out on Ligusticum and Crithmum.

The main part of the work is concerned with the longest stage in the life cycle of these species; as mature plants. The effect of temperature on the roots of such plants was investigated when the plants were in their winter condition. Chapter 4 deals with the way in which the respiration rate of the plants responded to a range of experimental temperatures after each of a variety of different temperature pretreatments. Root respiration rates are particularly important to a plant in its winter condition since there are few or no leaves to replenish carbohydrate reserves by photosynthesis, and even where leaves are present, light levels may not be adequate for a net gain of carbohydrate. Thus loss of stored carbohydrate by winter respiration is very important to the plant and may become critical if the temperature rises, leading to a higher rate of respiration and faster loss of reserves.

Some limited information on whole plant growth and response to temperature is recorded in Chapter 5, and Chapter 6 details analyses carried out to obtain data on the carbohydrate status of the roots after various temperature pretreatments. Chapter 7 serves as a final discussion of all the work together and this leads to the final conclusions.

CHAPTER 2

CORRELATION OF DISTRIBUTIONS OF COASTAL SPECIES WITH TEMPERATURE DATA

INTRODUCTION

The correlations between distribution and temperature for certain British species pointed out by Seddon (1971) and based largely on the distribution maps and isotherm overlays of the 'Atlas of the British Flora' (ed. Perring and Walters, 1976) were mentioned in Chapter 1. In this chapter, data have been extracted from the 'Atlas' for exclusively coastal British species which show northern and southern distribution limits, and these have been tabulated together with additional relevant information and world wide distribution where available: introduced, doubtfully native and very rare species have been omitted. This selection has resulted in a list of 43 species, of which, in Britain, 7 are northern (with a southern limit to their distribution) (Table 2.1) and 36 are southern (with a northern limit to their distribution), (Table 2.2).

Of the species listed in the Tables several were chosen for more detailed description as examples of the various categories of apparent temperature limitations on distribution, and all the five species studied in more detail in work of later chapters are included. The survey made in this chapter confirmed the validity of the prior selection of Ligusticum scoticum and Crithmum maritimum as good examples of northern and southern coastal species for investigation, and resulted in the later inclusion in the study of Mertensia maritima (northern), and Limonium binervosum and Glaucium flavum (both southern).

The species included in the Tables were selected by consideration of the distribution maps, and each distribution was subsequently compared with the isotherm overlays from the 'Atlas' showing the mean January and July temperatures and the mean February minimum temperature. The isotherms used in the preparation of the overlays in the 'Atlas' were however prepared

from Meteorological Office data for the years 1901-1930 (Meteorological Office, 1952) whereas the distributions on the maps are divided into pre- and post-1930 records. The latter have most relevance to present day distributions so the temperature data of the 'Atlas' are not strictly applicable to the mapped distributions. Temperature data are however available from records of individual meteorological recording stations for the years 1931-1960 (Meteorological Office, 1963) and the records for coastal stations have been used in the present study to make valid comparisons with the earlier isotherms at the coast. The 1931-1960 average temperatures for the coastal stations have been added to the isotherm maps, in Fig. 2.1 for January mean temperature, and in Fig. 2.2 for July mean temperature. Both these Figures fold out at the end of this chapter (after page 40). These maps show that the change in mean January temperature from the 1901-1930 to 1931-1960 periods has been a decrease of one or two degrees Fahrenheit[1] round the entire British coast with least change in western Scotland. A similar comparison of the July mean temperatures shows that the south and west coasts of Britain have, in the main, similar temperatures over the two periods while those on the east coast, from the Thames estuary to eastern Scotland, are consistently one or two degrees Fahrenheit higher in the 1931-1960 period. That temperature changes of this magnitude could be relevant to the present study is shown, for example, by Firbas and Losert (1949) who found that a $1^{\circ}C$ temperature decrease resulted in a 100 to 200m reduction in the level of the tree line, and by Lamb (1967) who pointed out that the limit for growth of vines moved 280 miles south for a $1^{\circ}C$ temperature drop.

In considering the influence of changes in seasonal temperature on distribution over a period of years it would be expected that changes which resulted in the loss of a species from an area would be more obvious than those which created a potentially extended range for a species since the colonisation of new areas by a plant is usually a much slower process than

1 - $^{\circ}F$ used since isotherms on overlays in the 'Atlas' are in $^{\circ}F$.

the loss of plants. Some changes in distribution between the periods 1901-1930 and 1931-1960 resulting from the coastal temperature changes discussed above are given in the detailed information on typical northern and southern species later in this chapter.

The following two sections, which cover northern and southern species respectively and include the relevant Tables and detailed descriptions referred to above, aim to correlate distribution and temperature for individual species in Britain. It has only been possible to attempt correlation between temperature and distribution for the European or world range of species in a few cases, partly through shortage of comparably detailed temperature data, and partly due to non-comparable methods for mapping of distribution used by different authors.

The sources used for compilation of the Tables are as follows:

ed. Perring and Walters, 1976 - all species.

Clapham, Tutin & Warburg, 1962 - all species.

ed. Tutin, 1964, 1968, 1972, 1976 - not monocotyledons.

Fitter, 1978 - not grasses, sedges etc.

Hultén, 1958 - various species.

Hultén, 1961 - monocotyledons.

Hultén, 1970 - dicotyledons.

Hultén, 1971 - various species.

Meusel, Jäger and Weinert, 1965 - various species.

NORTHERN SPECIES

A. General Considerations

The 7 'northern' species listed in Table 2.1 are those which, from data given in the 'Atlas of the British Flora' (ed. Perring and Walters, 1976) appear to have a southern limit to their distribution in Britain.

Of the northern species considered in the Table all except Elymus arenarius (see below) have the southern limit of their British distribution well defined by one of the July mean temperature isotherms. Ligusticum scoticum, for example, grows only where the July mean temperature is 59°F (15.0°C)[1] or less. Similarly, Mertensia maritima and Blysmus rufus are both limited by the 60°F (15.5°C) July mean in Britain.

Elymus arenarius does not appear to be a strictly northern species in Britain, though it does decrease markedly in frequency in the south of England. It has been widely planted as a sand binder and was once protected by law in both Scotland and England (Arber, 1934, p.339). It is clearly a northern species when its whole European distribution is considered, only extending south as far as the Cherbourg peninsula of northern France and continuing to 71°N in northern Scandinavia, (Bond, 1952). In France, the southern limit of this distribution correlates with a January mean of 6°C (42.8°F) and a July mean of 17°C (62.6°F) (temperature data from Wallen, 1970).

A more detailed description follows of Ligusticum scoticum and Mertensia maritima, both of which have distributions of the typically northern type in Britain. Both species are included in the studies of the present project described in later chapters.

1 - °F used with °C in brackets since this is the form used in the Atlas and its map overlays.

Table 2.1 : Coastal species with northern distributions in Britain; their habitat and world distribution

	Species[1]	Habitat	Distribution[2] and Notes
88/4	Cochlearia scotica Druce	Sands.	Scotland, Northumberland and N & W Ireland. J 45°, F 41°, Jul. 59/60°. Taxonomy doubtful.
306/1	Ligusticum scoticum L.	Rocks, cliffs, (sand).	Scotland, Northumberland, W. Ireland. J 43°, F 37°, Jul. 58/59°. European coast from Denmark to NW Russia. Circumpolar- Iceland, Greenland, N. America. L.s.ssp.hulteni in Alaska and Siberia. Naturalised in New Zealand. Leaves formerly used as vegetable.
402/1	Mertensia maritima (L.) S.F.Gray	Shingle (sand).	Lancashire and Aberdeenshire northwards. Formerly Northumberland, N and NE Ireland. J 42°, F 37/38°, Jul. 60° Denmark, Norway and NW Russia. Circumpolar - Iceland, Greenland, N. America to Alaska. Decreasing.
605/13	Juncus balticus Willd.	Dune slacks.	Lancashire and Fife northwards, but very local on west. J 42°, F 35/37°, Jul. 57/58°. Europe from Pyrenees to Faroes; Scandinavia & NW Russia. Iceland, N & S America, Japan, New Caledonia.
657/2	Blysmus rufus (Huds.) Link	Salt marsh.	S. Wales and Lincolnshire northwards. SW & E Ireland. J 45°, F 39°, Jul. 60°. Circumpolar
663/64	Carex maritima Gunn.	Damp hollows on fixed dunes.	E & N Scotland, Hebrides, Orkney and Shetland. J 41°, F 34/37°, Jul. 57/58°. Also inland in Europe in the Alps. C. maritima S.L. is circumpolar.

1 and 2 - Notes at end of table.

Table 2.1 (continued) : Coastal species with northern distributions in Britain; their habitat and world distribution

	Species[1]	Habitat	Distribution[2] and Notes
686/1	Elymus arenarius L.	Active sand dunes.	Local in S England, more frequent in N & E Britain. J $44^{\underline{0}}$, F $40^{\underline{0}}$, Jul. $61/62^{\underline{0}}$. To 71°N in Europe from N France and Belgium. Circumpolar - Iceland, Greenland, N. America and Siberia. Widely planted for dune stabilisation.

1. - The number preceding each species name is that used in the 'Atlas of the British Flora' and taken from Dandy (1958).

2a. - Limits are taken from 1901 - 1930 mean isotherms on map overlays in the 'Atlas of the British Flora', ed Perring and Walters, 1976.

b. - All limits are in $^{\underline{0}}$F and good correlations with distribution are underlined. A conversion table $^{\circ}$F to $^{\underline{0}}$C is given in Appendix 1.

c. - J is January mean temperature, F is February mean minimum temperature, Jul. is July mean temperature.

d. - Plants are <u>not</u> generally found where the relevant mean temperatures <u>exceed</u> the values given.

B. Detailed Consideration of Typical Species

a. Ligusticum scoticum L. (Haloscias scoticus(L.)Fr.), Family Umbelliferae. Scottish Lovage.

This plant, shown in photo 2.1, has bright green, biternate shiny leaves the petioles of which are often tinged dark red. The flowers of the compound umbels are usually white but may be pink, and are found from July to September in Scotland. The single seeded fruits are ripe by October or November and the plant dies back to ground level for overwintering. Growth recommences in late March or early April, as soon as there is a period of warm weather. The perennial rootstock is stout and long and the whole plant has a characteristic taste and smell when crushed. The leaves were formerly used as a vegetable or pot herb. In common with other perennial cliff plants Ligusticum is sensitive to grazing (Goldsmith, 1975).

L.scoticum is usually a plant of cliffs and rocky shores in the north of Britain, though it is occasionally found on shingle and sandy beaches and in such man made habitats as harbour walls etc. It is exclusively maritime throughout its range with the exception of Greenland where it is also found "in heath vegetation some distance from the sea", (Böcher et al, 1968).

In Britain L.scoticum is found northwards from Kirkudbright and Wigtownshire on the west coast and from Northumberland on the east coast. It is also found in north and west Ireland, and throughout Britain the southern limit of the distribution (Fig 2.3) shows a close correlation with the 58° or 59°F (14.4°C or 15.0°C) isotherms of July temperature for 1901 to 1930, (Fig. 2.2 after page 40).

The apparent slight retreat of the species northwards in Northumberland since 1930 shown in Fig 2.3 may be attributable to the increase in mean summer temperature on this part of the coast from 1901/1930 to 1931/1960 discussed on page 7 . In Fife where the species is near to the present southern limit in Britain, those plants which are found on northern and eastern facing parts of the shore appear to grow larger and to be generally more robust than those growing on shores with a southerly aspect.

In Europe L.scoticum is found northwards from Denmark to the Arctic circle though there are only 2 recorded stations on the Baltic, (ed Tutin et al, 1968). Overall it has a circumpolar distribution, being found in Iceland, Greenland, and North America, with a subspecies L.scoticum ssp. hulteni in Alaska and Siberia, (Hultén, 1958, map 276). L.scoticum is naturalised in New Zealand.

The distribution in Europe also shows reasonable correlation with the 15°C July mean temperature isotherm as mapped by Fitter (1978) (based on Wallen, 1970) for north and west Europe. The small scale of the map however makes accurate correlation difficult.

b. Mertensia maritima(L.)S.F. Gray; family Boraginaceae.
Oyster Plant, Sea Lungwort, Northern Shorewort.

This is a trailing plant (photo 2.2) with grey-green fleshy oval shaped leaves which have a salty fishy taste not unlike that of oysters. The pink or blue flowers are found from May to August and the resulting seeds are contained in black inflated nutlets. The black rootstock produces numerous new shoots each year which die back in late autumn to below ground level, and start to grow again in March or April (Scott, 1963a). The plant is able to tolerate being buried since it readily produces new shoots from its numerous underground buds.

Though usually found growing on shingle above the drift line, M.maritima is also occasionally found in sandy habitats. The distribution in Britain formerly extended from Anglesey and Norfolk northwards, and this was closely correlated with the 60°F (15.5°C) July mean isotherm for 1901 to 1930. However, the present distribution is much reduced and it now only extends as far south as Lancashire and Aberdeenshire, as shown in Fig 2.4. This reduced distribution can be directly attributed to the shift to higher summer temperatures from 1931 to 1960, especially on the east coast (shown in Fig 2.2 and discussed on page 7).

Overall the distribution of M.maritima is circumpolar and very

similar to that of Ligusticum scoticum. In Europe it is found on the coasts of Denmark, Norway and NW Russia though it is absent from the Baltic, (ed Tutin, 1972), and extends to Spitzbergen, Iceland, Greenland and North America. In NE Asia M.maritima is replaced by M.m.ssp asiatica though there is some doubt as to whether this should be a subspecies or a separate species (Hultén, 1958, map 275). In Alaska and Greenland M.maritima seems to be advancing northwards, very slowly, as the polar ice cap retreats and, as in Britain, it has become extinct in places in Europe and probably also in America and Asia, at the southern limits of its distribution (Scott, 1963a).

Photo 2.1: Ligusticum scoticum, a typical large plant at Portsoy, Banffshire, July 1976.

Photo 2.2: Mertensia maritima in typical shingle habitat, Skaill Bay, Orkney, June 1977.

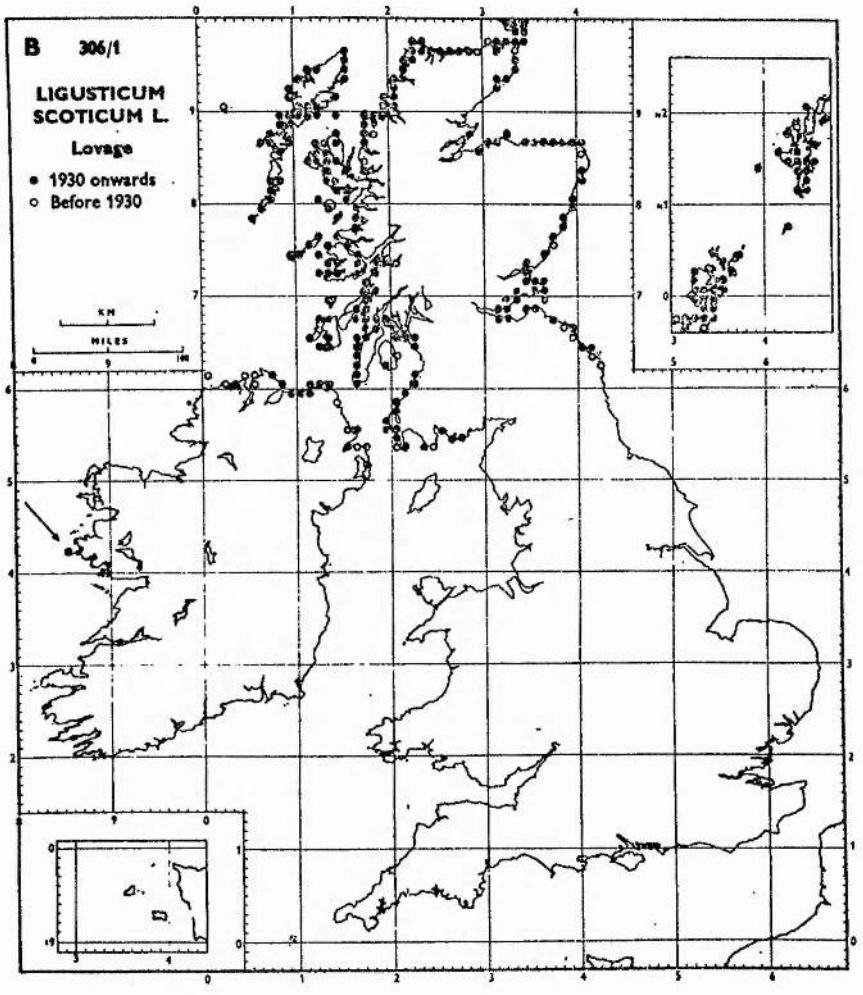


FIG. 2.3: Distribution of Ligusticum scoticum in Britain.

By permission of the Botanical Society of the British Isles, taken from their 'Atlas of the British Flora', and updated by the Biological Records Centre, Institute of Terrestrial Ecology, Monks Wood Experimental Station, Huntingdon.

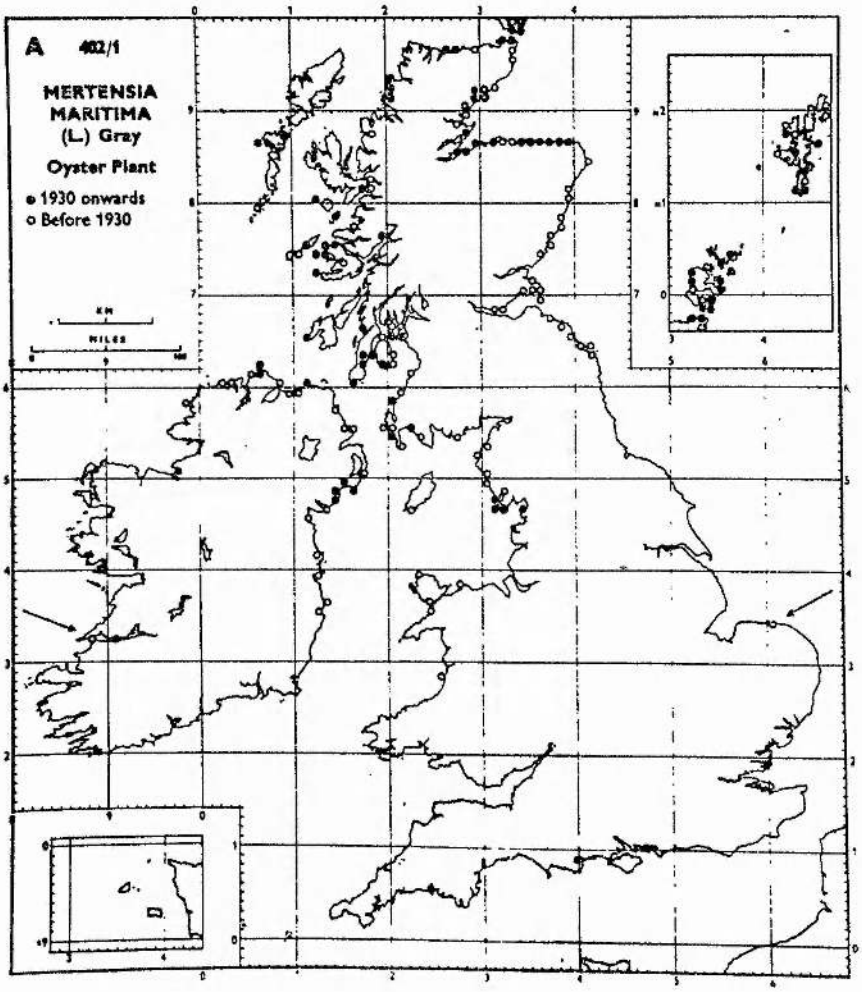


FIG. 2.4: Distribution of Mertensia maritima in Britain.

By permission of the Botanical Society of the British Isles, taken from their 'Atlas of the British Flora', and updated by the Biological Records Centre, Institute of Terrestrial Ecology, Monks Wood Experimental Station, Huntingdon.

SOUTHERN SPECIES

A. General Considerations

The 36 'southern' species listed in Table 2.2 are those which have a northern limit to their distribution in Britain as indicated by the maps of the 'Atlas of the British Flora' (ed. Perring and Walters, 1976).

There appear to be two clear groupings which can be made of the southern species on the basis of their British temperature limitations. The first category includes those species with a winter (January or February) temperature limitation, and the second, those with a summer temperature (July) limitation. Species with dual summer/winter temperature limitations and those with no clear temperature effect have been included in a third category.

The most extreme examples of plants which have a winter temperature limitation are those such as Lavatera arborea which will not tolerate frost (Okusanya, 1976). Others such as Crithmum maritimum, Calystegia soldanella and Eryngium maritimum all show restriction by winter temperature though it is not certainly known whether frost is damaging to these plants to the extent of limiting their distribution, though Malloch (1970) did find that seedlings of Crithmum maritimum were killed by frost which left older plants unaffected.

The second type of distribution seems to depend on summer temperatures, expressed here as July means, exceeding a certain critical level. Beta vulgaris ssp maritima, Halimione portulacoides, Limonium vulgare and Agropyron pungens all belong to this group and have limiting July mean temperature isotherms between 57°F (13.9°C) and 59°F (15.0°C), though the mechanism acting to restrict the plants is not clear for any one. A parallel might be drawn here with the temperature limitation on seed set in Cirsium acaulon in Derbyshire where it is at the northern limit of its range (Pigott, 1968).

The third grouping includes those plants which can only be described as generally southern with no clear single limitation, some of which may have a combined winter and summer temperature limitation. Species such as Cochlearia anglica, Inula crithmoides and

Puccinellia rupestris have no well defined temperature limits, while possible dual limitation by January and July mean temperatures is exhibited by Limonium binervosum (discussed below) and Euphorbia portlandica. Here there is a similarity with the observations of Iverson (1944) on the dual temperature limitations on Ilex aquifolium, Hedera helix, and Viscum album, and the good correlation between fossil finds of these plants and the projected contemporary climate for the relevant regions.

Two species from each of the first two categories, and three of the third group are considered below in detail as examples. The southern species, Crithmum maritimum, Limonium binervosum and Glaucium flavum, which were subjected to the experimental investigation described in later chapters are included.

Table 2.2 : Coastal species with southern distributions in Britain; their habitat and world distribution.

	Species[1]	Habitat	Distribution[2] and Notes
46/23	Ranunculus baudotii Godr.	Brackish streams and ditches.	Most of Britain but more frequent in the south. Ireland. J 39°, F 33/34°, Jul. 56°. To 64°N in the Baltic, not Norway.
61/1	Glaucium flavum Cranz	Shingle.	Argyll and Northumberland southwards. Ireland except north. J 39°, F 34/35°, Jul. 58°. Mainly Mediterranean, but north to Oslo. Naturalised frequently in central Europe. Poisonous root.
74/2	Rhaphanus maritimus Sm.	Rocks, cliffs, sand and drift line.	Argyll and Durham southwards. Ireland. J 39°, F 35°, Jul. 56/58°. Netherlands south to Mediterranean and Black seas.
75/1	Crambe maritima L.	Drift line and sand; also on shingle, rocks and cliffs.	Fife and Islay southwards. S & E Ireland. J 39°, F 34/35°, Jul. 58°. Oslo to N Spain; Baltic and Atlantic, also Black sea. Naturalised elsewhere from cultivation.
88/6	Cochlearia anglica L.	Muddy shores and estuaries.	Most of Britain but very rare in Scotland. Ireland. J 38/39°, F 33°, Jul. 56/57°. Atlantic and North sea coasts of Europe; Norway and Sweden to N Germany.
103/1	Matthiola incana (L.)R.Br.	Cliffs.	S. England, S. Wales, Isle of Wight. Doubtfully native in Durham (Holy Isle). S Ireland J 41/42°, F 36°, Jul. 62°. S. Europe and Mediterranean.

1 and 2 - Notes at end of table.

Table 2.2 (continued) : Coastal species with southern distributions in Britain; their habitat and world distribution.

	Species[1]	Habitat	Distribution[2] and Notes
121/1	Frankenia laevis L.	Sand or gravel above salt marsh.	Norfolk to Isle of Wight. J 39°, F 34°, Jul. 60/61°. W Europe and W Mediterranean to Italy. Azores.
143/3	Spergularia rupicola Lebel	Cliffs and rocks.	Islay to Isle of Wight. Ireland. J 41/42°, F 34°, Jul. 57°. W Europe, France to Spain and Portugal.
155/1	Beta vulgaris L. ssp.maritima(L.)Thell.	Shores.	Most of Britain but infrequent in Scotland. J 39°, F 34°, Jul. 57/58°. Mediterranean, S & W Europe to S Sweden. B.v.ssp.vulgaris is cultivated and may be confused.
157/1	Halimione portulacoides (L.)Aell.	Salt marsh, higher levels.	Cumberland and Northumberland southwards. S & E Ireland. J 39°, F 34°, Jul. 58/59° Denmark south, Mediterranean.
158/2	Suaeda fruticosa Forsk. (S.vera J.F.Gmelin)	Shingle above mean high water springs.	Lincolnshire to Dorset, local. J 39°, F 34°, Jul. 60°. South from France to W Mediterranean. Madeira, Canaries.
160/1	Salicornia perennis Mill.	Gravel and salt marsh.	Lincolnshire to Devon. SE Ireland. J 39°, F 34°, Jul. 59/60°. France to Algeria.
160/5	Salicornia pusilla Woods	Salt marsh, drier parts.	Lincolnshire to S Wales. South Ireland. J 39°, F 34°, Jul. 60° NW France.

1 and 2 - Notes at end of table.

Table 2.2 (continued) : Coastal species with southern distributions in Britain; their habitat and world distribution.

	Species[1]	Habitat	Distribution[2] and Notes
164/1	Lavatera arborea L.	Rocks or waste ground to 500 feet.	Ayrshire, Isle of Man, N Wales to Sussex. S & W Ireland. Introduced on East Coast of England. J 40/41°, F 37°, Jul. 58°. France to Mediterranean and N Africa; Canaries. Seedlings are frost sensitive.
272/1	Eryngium maritimum L.	Sand and shingle.	S Yorkshire and Hebrides south. Formerly on east coast of Scotland. J 39/40°, F 35°, Jul. 56/58°. S Scandinavia to Portugal; Mediterranean and Black seas.
298/1	Crithmum maritimum L.	Cliffs and rocks, also shingle and sand.	Hebrides south to Suffolk. Ireland. J 40°, F 35°, Jul. 55/58°. Mediterranean and Black seas, Atlantic coast to France. Leaves used for a pickle.
319/12	Euphorbia portlandica L.	Sand and young dunes.	Wigtownshire to Sussex. Ireland. J 40/41°, F 35/36°, Jul. 57° France to Gibraltar.
319/13	Euphorbia paralias L.	Sand and mobile dunes.	Wigtownshire to Norfolk. Ireland. J 39/40°, F 35°, Jul. 57/58°. Atlantic coast from Holland to Morocco.
320/2	Polygonum raii Bab. (P.oxyspermum Meyer Bunge ex Ledeb.)	Sand or fine shingle above mean high water springs.	Hebrides to Kent. S & W Ireland. Local throughout. J 39/40°, F 35°, Jul. 55/58°. NW France to Arctic Russia. Eastern N America. Taxonomy confused, not truly southern though it appears so in Britain.

1 and 2 - Notes at end of table.

Table 2.2 (continued) : Coastal species with southern distributions in Britain; their habitat and world distribution.

	Species[1]	Habitat	Distribution[2] and Notes
325/16	Rumex rupestris Le Gall	Cliffs, rocks and dune slacks.	Dorset to S Wales & Anglesey. J 42°, F 38°, Jul. 59/60°. W. France and NW. Spain.
365/1	Limonium vulgare Mill.	Salt marsh.	Fife and Dumfries southwards. J 39°, F 34/35°, Jul. 58/59°. SW Sweden to Mediterranean and N. Africa. N. America.
365/2	Limonium humile Mill.	Salt marsh.	Northumberland and Dumfries southwards. Ireland. J 39/40°, F 35°, Jul, 57/59°. S. Scandinavia to Brittany. Very closely related species L.nashii in N America.
365/5	Limonium binervosum (G.E.Sm.)C.E.Salmon	Cliffs and rocks, also stabilised shingle.	South from Lincolnshire and Wigtownshire. Ireland. J 39/40°, F 35°, Jul. 57/58° Atlantic coasts of France, Spain and Portugal.
406/4	Calystegia soldanella (L.)R.Br.	Sand, dunes and shingle.	From Hebrides and Berwickshire southwards. Ireland. J 39/40°, F 34/35°, Jul. 56/58°. Denmark to Mediterranean, Black and Caspian seas. West coasts of N & S America. Australia, New Zealand, China, Korea and Japan. Disjoint world distribution.
512/5	Inula crithmoides L.	Salt marsh, shingle, cliffs and rocks.	Wigtownshire to Essex. S & E Ireland. J 40°, F 35°, Jul. 58/61°. France to Mediterranean and W Asia. Inland in E Spain.

1 and 2 - Notes at end of table.

Table 2.2 (continued) : Coastal species with southern distributions in Britain; their habitat and world distribution.

	Species[1]	Habitat	Distribution[2] and Notes
535/7	Artemisia maritima L.	Dry salt marsh, sea walls.	Angus and Cumberland southwards. E & W Ireland. Local. J 38/40°, F 33/34°, Jul. 58°. W France to Sweden. Highly polymorphic, many closely related species around Mediterranean and European coasts.
605/15	Juncus acutus L.	Sand, dune slacks; also salt marsh.	Caernaryonshire to Norfolk. S & E Ireland. J 39/40°, F 35°, Jul. 59/60°. N France to Mediterranean and N Africa. California, S America, S Africa. Inland in Spain and Asia.
670/7	Festuca juncifolia St.-Amans	Dunes.	S. Wales and Devon to Angus. J 39°, F 34°, Jul. 59/60°. W. Europe.
672/1	Vulpia membranacea (L.)Dum.	Dunes, in hollows.	Norfolk to Lancashire, E Ireland. J 39°, F 34°, Jul. 59° W Europe and Mediterranean.
673/5	Puccinellia rupestris (With.)Fern.&Weath.	Clayey and stony shores by brackish ditches.	S Yorkshire to S Wales, local. E Ireland. J 39°, F 34/35°, Jul. 58/59°. W Europe, Syria.
685/4	Agropyron pungens (Pers.)Roem.&Schult.	Dunes and salt marshes.	N. Yorkshire and Cumberland southwards. N & E Ireland. J 39°, F 35°, Jul. 59°

1 and 2 - Notes at end of table.

Table 2.2 (continued) : Coastal species with southern distributions in Britain; their habitat and world distribution.

	Species[1]	Habitat	Distribution[2] and Notes
707/5	Phleum arenarium L.	Dunes and sandy fields.	Aberdeenshire and Argyll southwards. Ireland except SW. J 39°, F 34°, Jul. 57° S. Sweden to Mediterranean, local.
708/5	Alopecurus bulbosus Gouan	Grassy salt marshes.	Gloucestershire to Lincolnshire. J 39°, F 35°, Jul. 60° W Europe, France to Italy and Algeria.
714/1	Parapholis strigosa (Dum.)C.E.Hubbard	Salt marsh turf.	Ayrshire and Lothian southwards. Ireland. J 39°, F 34°, Jul. 57/58°. W Europe.
716/1	Spartina maritima (Curt.) Fernald	Tidal mud flats.	S Devon to Lincolnshire. J 39°, F 35°, Jul. 60°. SW Europe.
716/1x2	Spartina x townsendii H. & J. Groves	Tidal mud flats.	S England, planted elsewhere. Ireland. J 39°, F 34°, Jul. 58° Much planted as a mud binder in all temperate regions.

1. - as for Table 2.1 (page 11)

2. - a, b, and c as for Table 2.1
d, Plants are not generally found where the mean temperatures of limits are less than the values given.

B. Detailed Considerations of Typical Species

1. Winter Temperature Limitation

a. Crithmum maritimum L., Family Umbelliferae. Rock Samphire.

The fleshy finger-like leaves of Crithmum are much divided and of a bright green to grey-green colour. The plant produces its yellow green compound umbels from July to September and the somewhat corky single seeded fruits are ripe in late October or early November. Seed set in Crithmum appears to be dependent on summer temperature conditions. Okusanya (1976) found that viable seed was only obtained in North Wales and Cumbria, near the limits of its range, after the particularly warm summer of 1975, though further south (South Wales and SW England) viable seed was set in every year studied (1973 to 1975). Many of the leaves of the plant die back for the winter leaving the long woody rootstock underground. The whole plant has a characteristic taste and smell and the leaves used to be made into a pickle with spices and vinegar. Photo 2.3 shows the plant in November.

C.maritimum is found on cliffs and rocky shores in southern and western Britain, replacing Ligusticum scoticum in the south and overlapping with it in south and west Scotland. The distribution of C. maritimum is shown in Fig. 2.5 and this can be compared with Fig 2.3 to show the complementary distribution of L. scoticum. Crithmum, however is absent from much of the east coast of England only reaching a few isolated localities in Suffolk. It is found all round the Irish coast. This distribution in Britain coincides very closely with the 40°F (4.4°C) January mean isotherm and also with the mean February minimum isotherm of 35°F (1.7°C) but it is not clear which of these low temperatures is likely to have the most detrimental effect on the survival and distribution of the plants. From the information given in Fig 2.5 it would appear that the distribution of C. maritimum has changed little between the two periods, 1901/1930 and 1931/1960, but it should be noted that the greatest changes in winter temperatures between the two periods (Fig. 2.1 and discussed on page 7) are on the east coast, north of the limits for Crithmum.

Photo 2.3: Crithmum maritimum on limestone cliffs, Great Orme, Gwynedd, November 1976.

In Europe, Crithmum is found on the Atlantic coast from France southwards, and all around the Mediterranean sea, (ed. Tutin, 1968).

b. Spergularia rupicola Lebel, family Caryophyllaceae, Cliff Sand-spurrey.

This trailing perennial has a woody, stout rootstock and linear acute fleshy leaves. The flowers are pink and are found from June to September, and the small black seeds, contained in a capsule, become ripe by November. The plant seems to be easily propagated from seed since it 'appeared' in the collected sample of Crithmum maritimum plants in the absence of any whole plant material.

In Britain, S. rupicola is found on walls, cliffs and rocks on the south and west coasts from Hampshire to the Outer Hebrides and around most of the Irish coast, as shown in Fig. 2.6. The 40°F and 41°F (4.4° and 5.0°C) January mean temperature isotherms bound the limits of the distribution of the plant in Britain with a good degree of correlation, and summer temperature appears to play no part in this. Okusanya (1976) included this plant in his study and suggested that frost intolerance was limiting its distribution.

In Europe, S. rupicola is found from North France southwards to Spain and Portugal. (ed. Tutin, 1964).

2. Summer Temperature Limitation

a. Halimione portulacoides (L.) Aell., family Chenopodiaceae, Sea Purslane.

This small shrub has decumbent stems with ascending branches and mealy, variably shaped leaves. The flowers make up a dense compound inflorescence and are found from July to September. The fruit is ripe between September and November.

In Britain, H. portulacoides is found in the higher part of salt marshes from Northumberland and Wigotwnshire southwards and around the south and east coasts of Ireland. This distribution is shown in Fig 2.7

and the 58°F (14.4°C) July mean isotherm is found to be limiting with good correlation. Chapman (1950) suggested a limitation by 60°F (15.5°C) July temperature in Britain and by 60°F August temperature in Europe where the plant is found on all coasts south of Denmark and also on the Baltic sea. He also suggested that H. portulacoides is restricted to areas where the average annual temperature is greater than 50°F (10.0°C). It is also found in North Africa, Asia Minor and South Africa, and has been introduced to North America.

b. Limonium vulgare Mill., family Plumbaginaceae, Sea Lavender.

The blue-purple flowers of this salt marsh plant are found from July to October on the large spreading inflorescence which arises on a single upright peduncle from the rosettes of elongate leaves. The plant is a perennial and has a deep tap root and branched woody stock.

The distribution of L. vulgare in Britain extends southwards from Fife and Dumfries as shown in Fig. 2.8, and it has its limit between the 59°F and 58°F (15.0°C and 14.4°C) July mean isotherms, Fig. 2.2 (page 42). It is absent from Ireland. Boorman (1967) states that there is little evidence of damage by adverse winter weather in Britain but he points out that the plant has a narrow altitudinal range of 1m and suggests that this may limit the distribution. A further possibility is that L. vulgare has a minimum temperature for successful seed set in the summer, which may explain the lack of limitation by winter temperatures. The plant is susceptible to trampling and grazing and is intolerant of pollution and drought, however, L. vulgare was observed by Boorman (1967) to be little affected by the severe winter of 1962/63.

The overall distribution of the plant is West and South Europe, to S.W. Sweden (ed. Tutin 1972). It is also found in North Africa and North America.

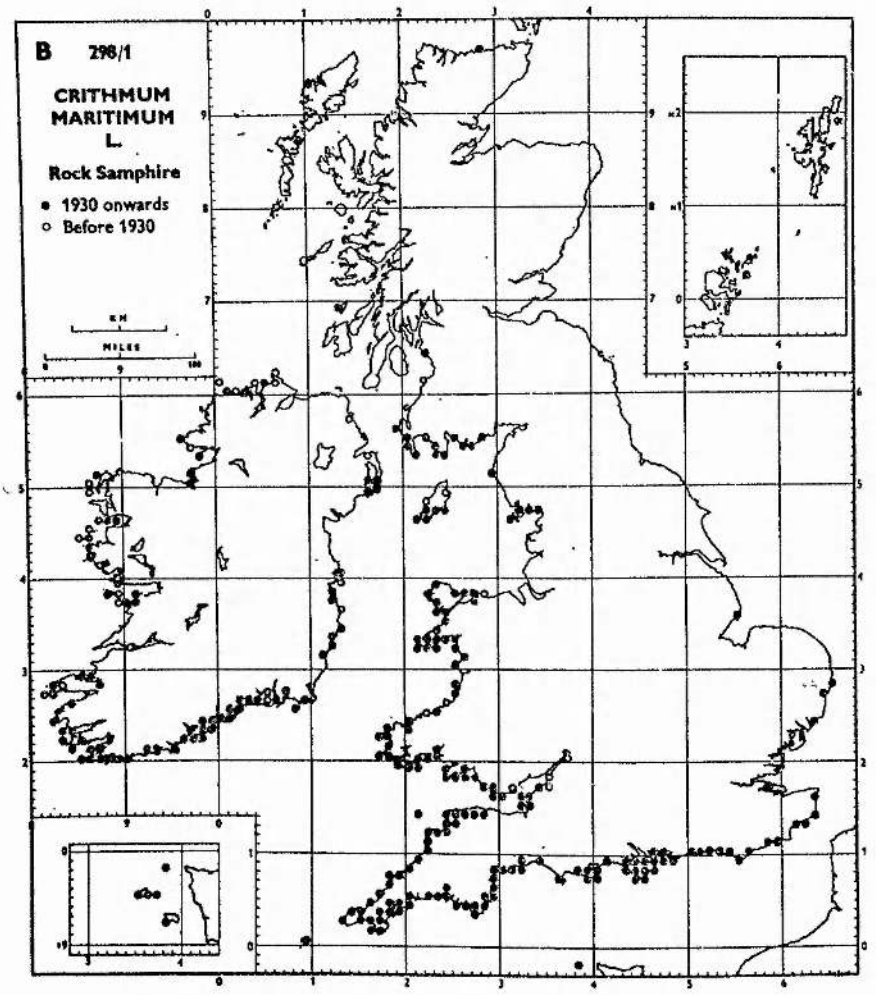


FIG. 2.5: Distribution of Crithmum maritimum in Britain.

By permission of the Botanical Society of the British Isles, taken from their 'Atlas of the British Flora', and updated by the Biological Records Centre, Institute of Terrestrial Ecology, Monks Wood Experimental Station, Huntingdon.

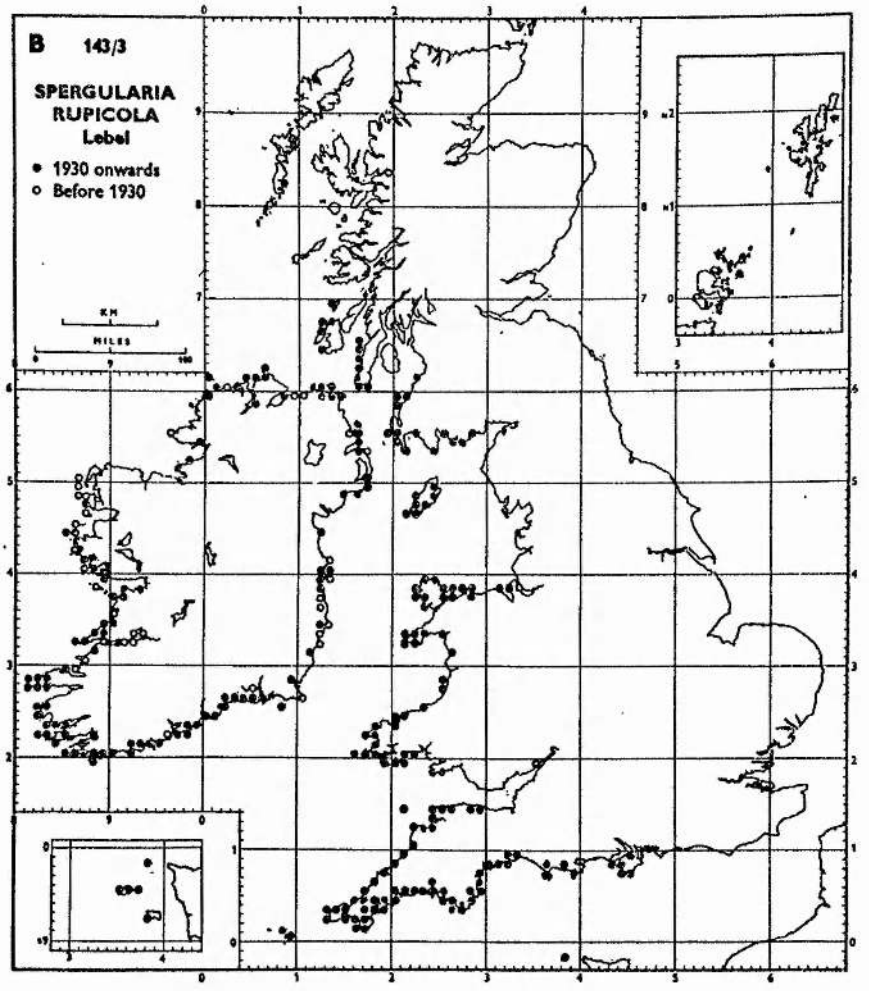


FIG. 2.6: Distribution of Spergularia rupicola in Britain.

By permission of the Botanical Society of the British Isles, taken from their 'Atlas of the British Flora', and updated by the Biological Records Centre, Institute of Terrestrial Ecology, Monks Wood Experimental Station, Huntingdon.

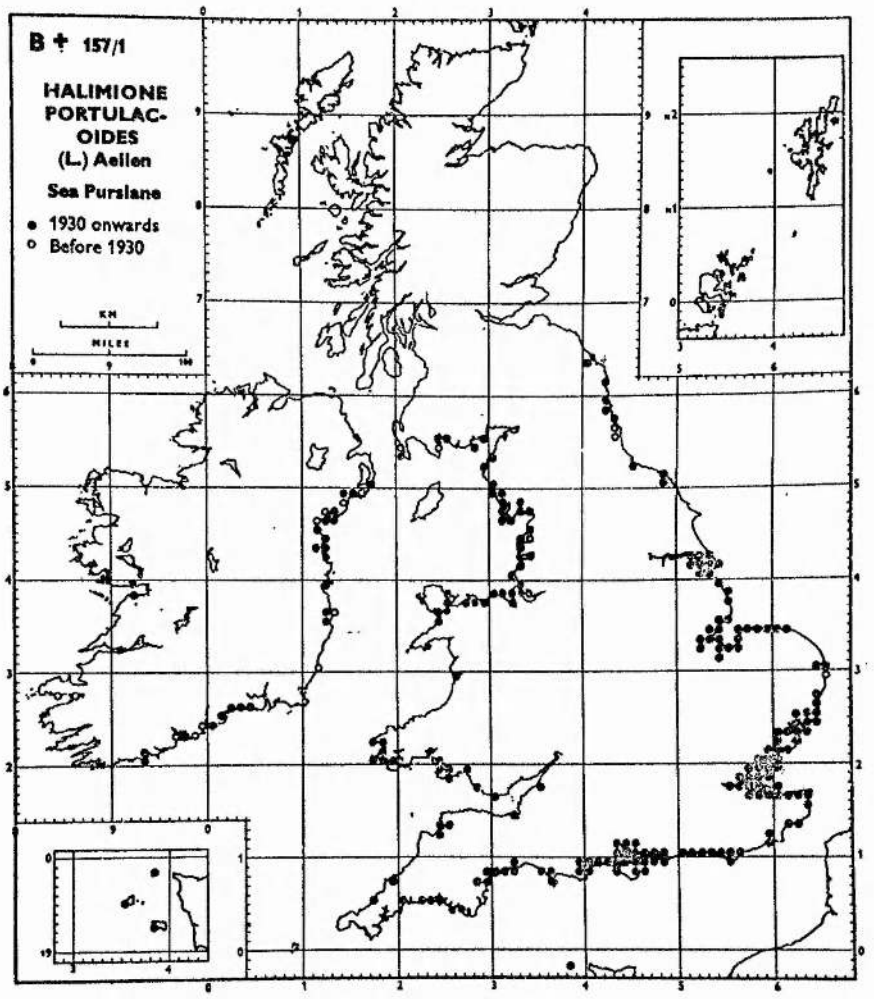


FIG. 2.7: Distribution of Halimione portulacoides in Britain.

By permission of the Botanical Society of the British Isles, taken from their 'Atlas of the British Flora', and updated by the Biological Records Centre, Institute of Terrestrial Ecology, Monks Wood Experimental Station, Huntingdon.

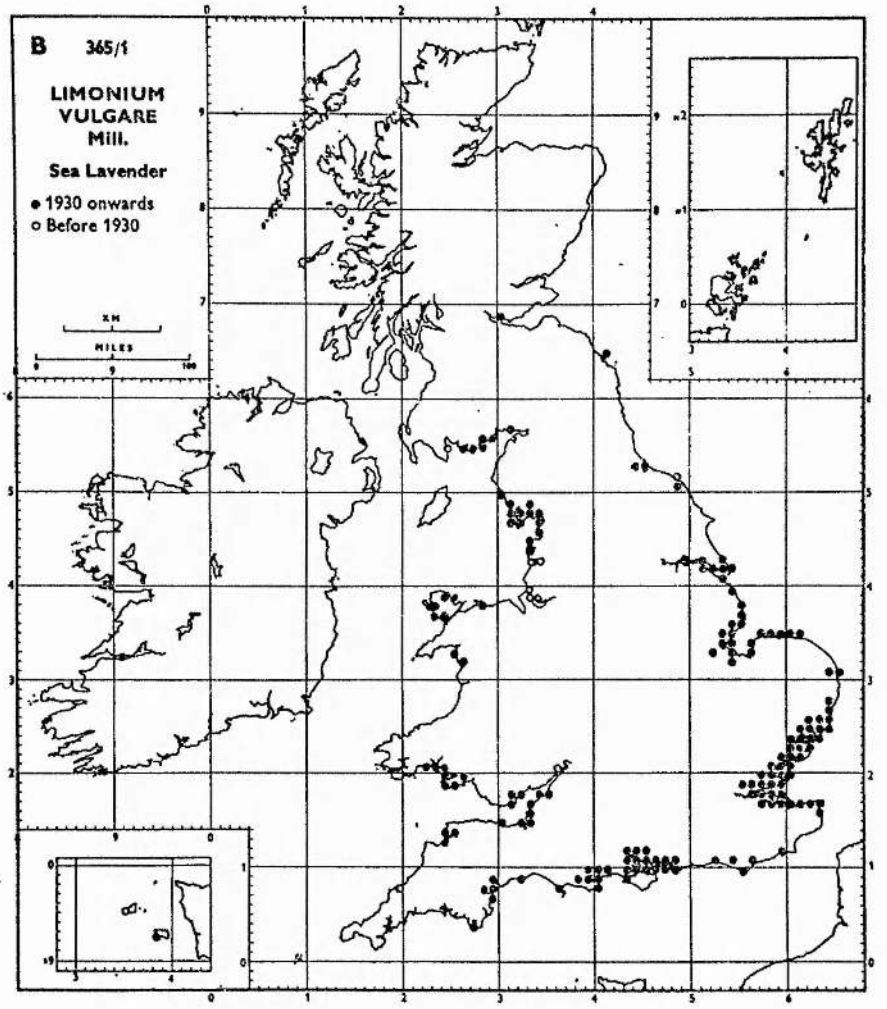


FIG. 2.8: Distribution of Limonium vulgare in Britain.

By permission of the Botanical Society of the British Isles, taken from their 'Atlas of the British Flora', and updated by the Biological Records Centre, Institute of Terrestrial Ecology, Monks Wood Experimental Station, Huntingdon.

3. Winter and Summer Temperature Limitations

a. Limonium binervosum (G.E.Sm.) C.E. Salmon, family Plumbaginaceae, Rock Sea-Lavender.

This plant is very similar to L. vulgare (2, above) but is a generally smaller plant with leaves of very variable shape. The small violet-blue flowers are found from July to September and result in small narrow reddish-brown seeds.

L. binervosum grows on cliffs, rocks and stabilised shingle in the southern part of Britain from Wigtownshire round to Lincolnshire and around much of the Irish coast as shown in Fig. 2.9. It is also found on the Atlantic coasts of France, Spain and Portugal in Europe.

In Britain, though the distribution is undoubtedly southern it shows no direct limitation by winter or summer temperatures alone. The limits on both west and east coasts can apparently be defined by using a combination of the January and July mean temperatures. It is found where a simple addition of these figures yields a value of about 100°F or 20.0°C or greater. For example the limit in Northern Ireland has a January mean of 43°F (6.1°C) and a July mean of 57°F (13.9°C), that in south west Scotland a January mean of 41/42°F (5.0/5.5°C) and a July mean of 58/59°F (14.4/15.0°C), and that in Lincolnshire a January mean of 39/40°F (3.9/4.4°C) and a July mean of 60°F (15.5°C),(taken from the isotherms on Figs. 2.1 and 2.2).

The fact that L. binervosum is found only on the Atlantic coasts of Europe suggests that there may also be a combined temperature maximum limit, though Boorman (1967) states that it can withstand considerable drought.

b. Glaucium flavum Cranz, family Papaveraceae, Yellow Horned-poppy.

This is a perennial or biennial plant with a rosette of glaucous divided leaves and a deep stout tap root which is poisonous, (Polunin, 1969). The flowers which are large with four roundish yellow petals are found from June to September and the resulting capsules are long and horn shaped, giving the plant its name.

As shown in Fig. 2.10, the post-1930 distribution of G. flavum in Britain extends from Argyll and Northumberland southwards, though it was

formerly found as far north as Shetland, and around all but the northern coasts of Ireland. Both summer and winter temperature appear to have an influence on the distribution of the plant which is approximately limited in the north by the 57/58°F (13.9/14.4°C) July mean isotherms. However, on the east coast alone, the winter temperature appears to play an important part in the limitation of its distribution, since before 1930 Glaucium extended to where the mean January temperature was 39/40°F (3.9/4.4°C) and since then its range has receded in keeping with the general changes in winter temperature discussed on page 7 and shown in Fig. 2.1. The present northern limit of this plant on the east correlates with January mean temperatures for 1931/1960 of close to 39°F (3.9°C).

In Europe, G. flavum is found from southern Norway and Sweden southwards to its main centre of distribution, the Mediterranean sea. It extends up some rivers in France, Spain and Portugal and is found naturalised inland in central Europe and the east Mediterranean (ed. Tutin 1964). The plant is naturalised in New Zealand and North America.

Scott (1963b) observes that frost may cause some blackening of the leaves but that slight frost has little effect and also that the plant is very resistant to drought though this may affect size and performance. The observation on frost susceptibility gives some support to the indications of cold intolerance suggested by the temperature data discussed above. However, despite the severe weather in January and February 1979, Glaucium flavum plants in St. Andrews Botanic Garden still retained healthy looking inner leaves and buds up to March 1979, though most of the outer leaves had died back. This behaviour may not reflect accurately that of the plant under more natural conditions.

c. Inula crithmoides L., family Compositae, Golden Samphire

The stem and leaves of this plant are glabrous and fleshy and borne on a branched woody stock. The flower heads have numerous ray-florets which are golden yellow around the orange-yellow disc-florets. The plant is in flower in July and August.

I. crithmoides is found on salt marshes, shingle banks, cliffs and rocks around the coast of Britain from Essex to Wigtownshire as shown in Fig. 2.11 and also in south and east Ireland. In Europe it is found from France to the Mediterranean and west Asia, and also inland in east Spain (ed. Tutin 1976). There are no clear cut limitations to the distribution in Britain from the temperature data. The nearest close limitation being by the 35/36°F (1.7/2.2°C) February minimum isotherm. On the west coast there is also good correlation between the July mean isotherms of 58°F and 59°F (14.4°C and 15.0°C) and the distribution of I. crithmoides, however, on the east coast 62°F (16.7°C) is the limiting July temperature. No firm conclusions can be drawn from this on the part which temperature plays in restricting the distribution of this plant, and the only clear fact is that I. crithmoides is a southern species in Britain. Okusanya (1976) suggests that seed set is possibly a limiting factor in the north for this plant.

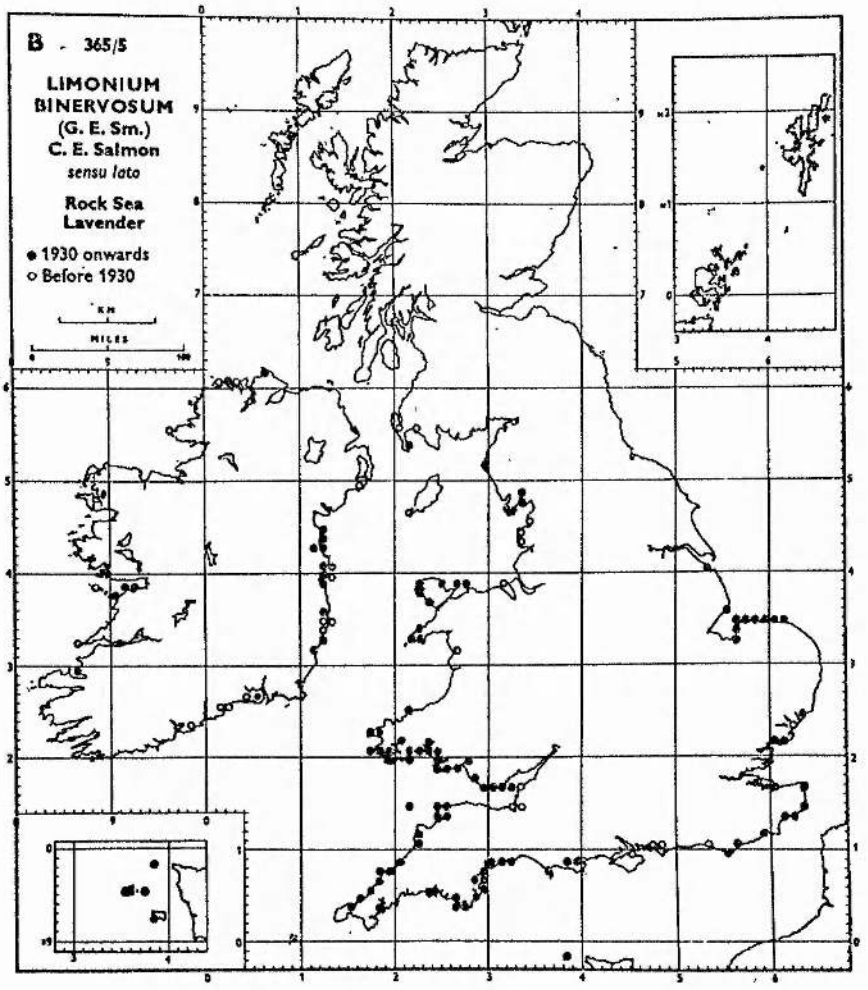


FIG. 2.9: Distribution of Limonium binervosum in Britain.

By permission of the Botanical Society of the British Isles, taken from their 'Atlas of the British Flora', and updated by the Biological Records Centre, Institute of Terrestrial Ecology, Monks Wood Experimental Station, Huntingdon.

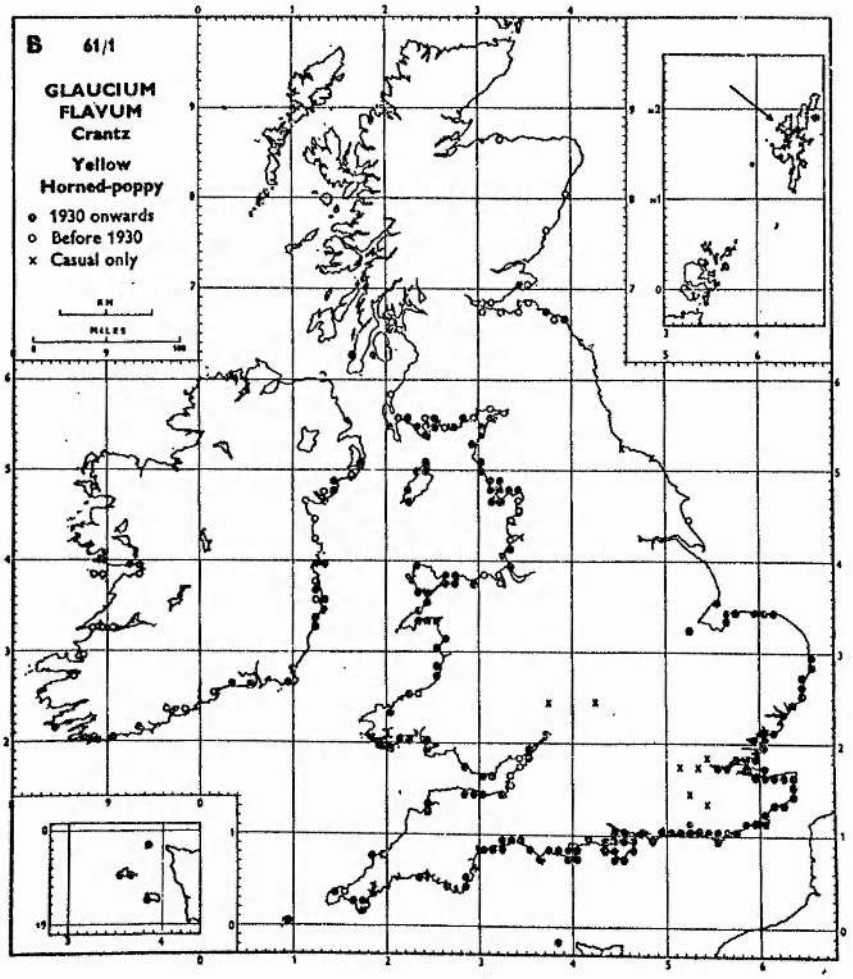


FIG. 2.10: Distribution of Glaucium flavum in Britain.

By permission of the Botanical Society of the British Isles, taken from their 'Atlas of the British Flora', and updated by the Biological Records Centre, Institute of Terrestrial Ecology, Monks Wood Experimental Station, Huntingdon.

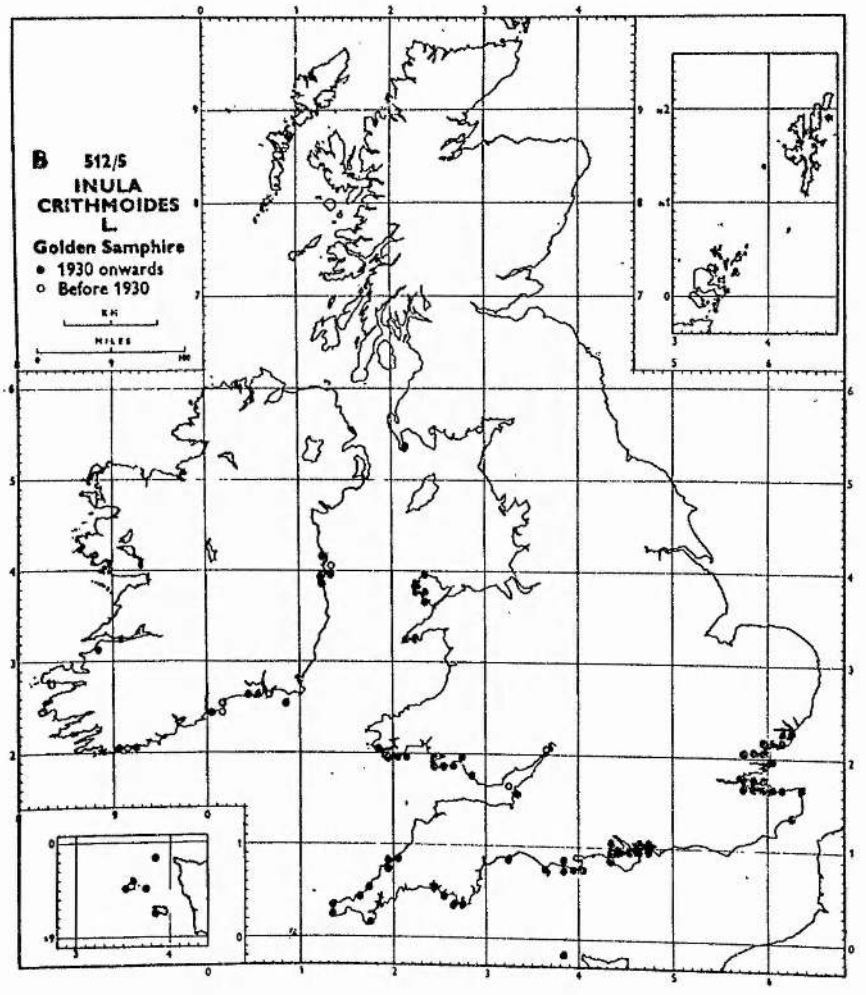


FIG. 2.11: Distribution of Inula crithmoides in Britain.

By permission of the Botanical Society of the British Isles, taken from their 'Atlas of the British Flora', and updated by the Biological Records Centre, Institute of Terrestrial Ecology, Monks Wood Experimental Station, Huntingdon.

CONCLUSION

The studies of distribution in relation to temperature for British coastal species in this chapter have shown that the limitation on northern species is probably an intolerance of higher summer temperatures, and their southern limits correlate well with July mean isotherms. The two most readily obtainable and interesting species in this category were chosen for the physiological studies of later chapters: *Ligusticum scoticum* because it was reasonably plentiful around St. Andrews, and *Mertensia maritima* because some material at least was obtainable.

The northern limits of the southern species fall into the various categories discussed in the chapter, and the species used in later study were chosen from those considered in detail for a variety of reasons. *Crithmum maritimum*, a species limited by winter temperature, was chosen because it was of the same family as *Ligusticum*, complementary in distribution to it, and was fairly readily obtainable. *Glaucium flavum* and *Limonium binervosum* were chosen because some material was available in Botanic Gardens at St. Andrews and Edinburgh for these rather less common species, thus avoiding much collecting from the wild.

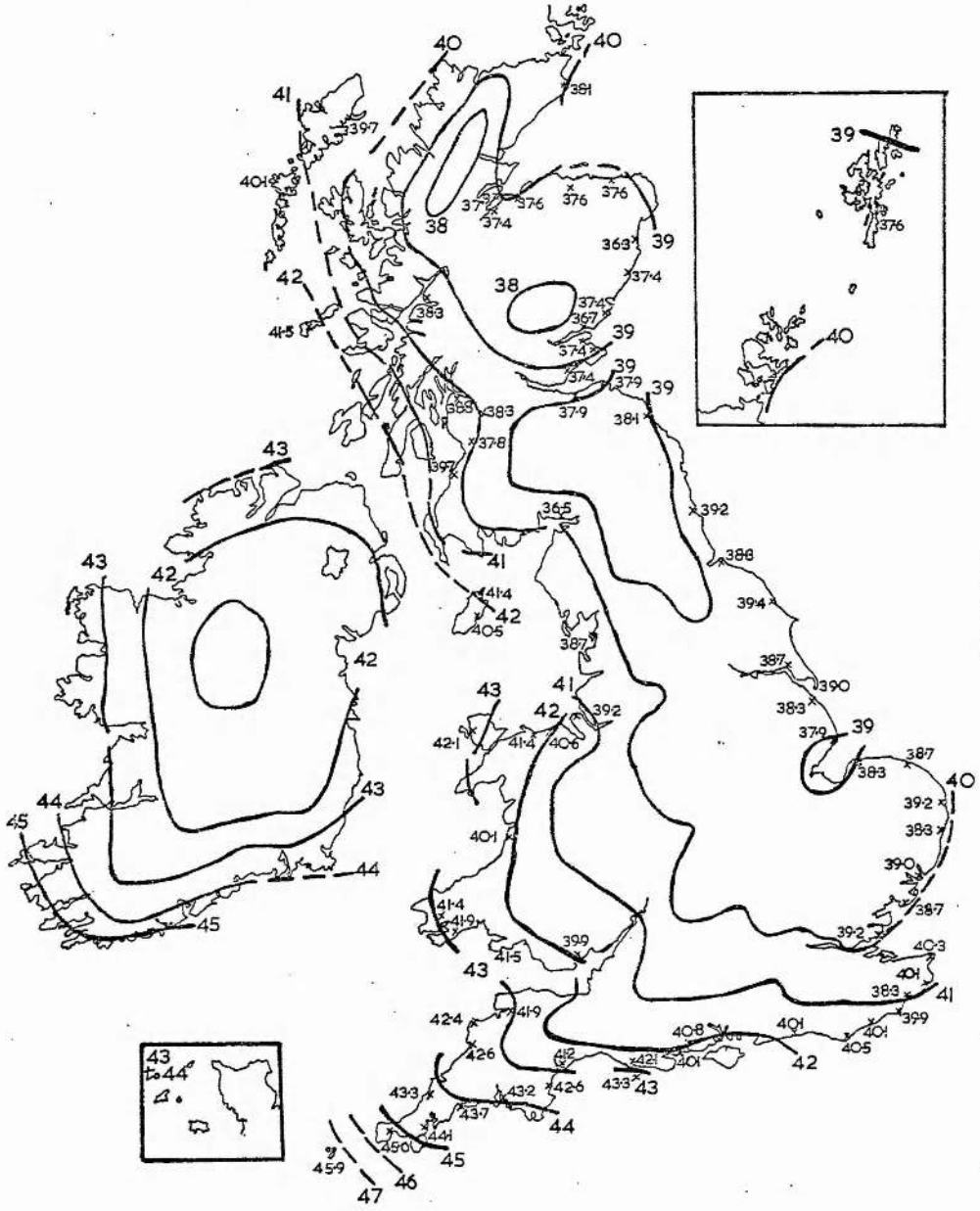


FIG. 2.1: Comparison of the isotherms for mean January temperature

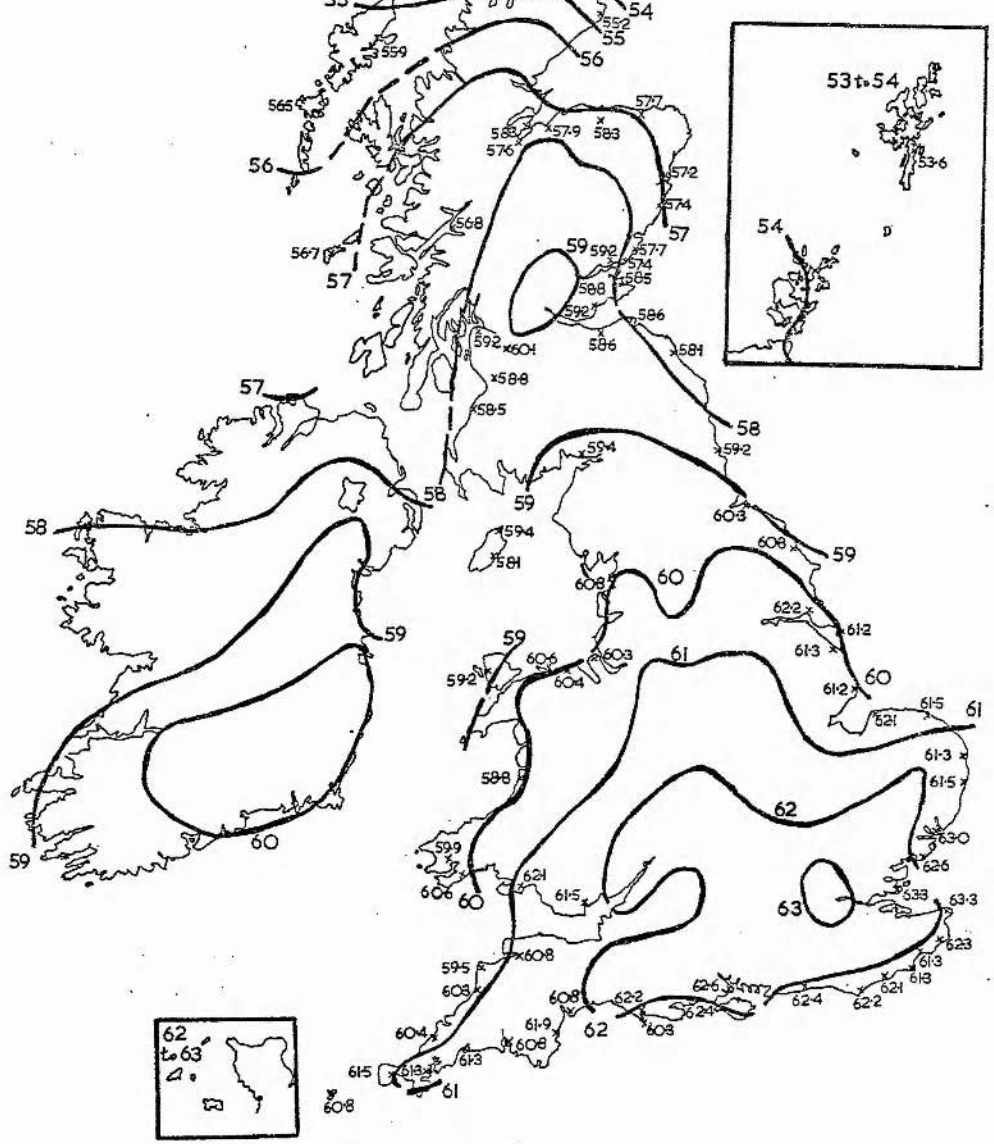


FIG. 2.2: Comparison of the isotherms for mean July temperature for the years 1901 to 1930 (large figures) and the mean temperatures for coastal meteorological stations for the years 1931 to 1960 (small figures): each coastal station is marked by an x. All temperatures are in degrees Fahrenheit since this was used for the original isotherms. A conversion table from °F to °C is in Appendix 1.

CHAPTER 3

THE EFFECT OF TEMPERATURE ON SEED GERMINATION OF COASTAL SPECIES

INTRODUCTION

Seed of any species has its own individual requirements before it will germinate, and optimum germination often requires a very closely defined set of conditions. These conditions are of great importance to the plant in an ecological sense: they may play a direct part in determination of the distribution of the plant, especially in annual or short lived species, or they may have a subsidiary role in longer lived or essentially vegetative species.

Much work has been done previously, characterising the germination requirements for a variety of species, especially the southern and cultivated ones studied by Thompson (1973, 1974a, 1974b) which include Agrostemma githago L., Lycopus europaeus L., Silene dioica (L.) Clairv., Clarkia unguiculata Lindl. and Apium graveolens L.. In all these species temperature plays a direct regulatory role on germination, though in different characteristic ways. Some need constant temperatures, some widely fluctuating ones, and others require a low temperature period before any germination will occur. In some cases however, the temperature requirements for germination may be less demanding or they may change with varying lengths and conditions of storage. Light has also been found to affect the germination of some species as shown, for example, by the work of Popay and Roberts (1970a & b) on Senecio vulgaris L. and Capsella bursa-pastoris (L.) Medic. Information on light requirements has most practical relevance for crop plants where the seed may be buried, in contrast to the natural state where the seed usually just falls to the ground.

There are so many different categories into which seed germination types can be grouped that generalisations are usually of very little use. Categorisation may be applicable where groups

such as crop plants are being considered or when there is some specific purpose to the investigation, as in the present project, where comparison of the germination characteristics in relation to distribution and its limitation by temperature are being made.

Several authors have determined germination characteristics of northern and southern plants and have related these to their distribution limits with some important conclusions. Billings and Mooney (1968) have pointed out that arctic plants often have a higher temperature optimum for germination - between 20° and 30°C - than alpine or temperate plants. This is of ecological importance to the plants since these temperatures are only reached in arctic latitudes in late spring and early summer so that the establishment of seedlings is possible before the onset of winter. Conversely, Thompson (1970) observes that Mediterranean species show a marked adaptation to rapid germination at low temperatures, which reflects the advantages of seedling establishment in the cooler part of the year when drought is not a problem. Seed of southern species tends to germinate in spring or autumn or in both seasons, and there may even be further safeguards against a 'bad' season such as a gradual release of seed dormancy over several years.

Where a plant has a very wide range of distribution it is often found that there is a moderate degree of ecotypic variation which is also reflected in the temperature requirements for germination of seed from different ecotypes. Thompson (1973) found variations between different populations of Agrostemma githago in their germination responses, though these were only statistically significant between populations from the extreme ends of the distributional range.

Of the plants investigated in the present study, the germination of both Ligusticum scoticum and Crithmum maritimum has previously been studied by Okusanya (1976), though this work only came to my attention after completion of most of the experimental work described in this chapter and is complementary to it. The findings in relation to temperature are similar to those of the present study; however,

additional information on germination performance in sea-water was also obtained with particular reference to Crithmum (Okusanya, 1977).

Also indirectly relevant to the present study are the observations collected by Ridley (1930) on the ability of fruits and seeds of some of the species included to float and retain their viability in sea-water. The mericarps of Crithmum float for up to one year, and those of Ligusticum for two to three months; the inflated nutlets of Mertensia maritima also float and all three species may be effectively dispersed in this way with the associated influence on their distribution.

Morphological and temperature data on the germination of Mertensia and Glaucium flavum given by Scott (1963a and 1963b) adds little directly to the present study and is referred to where relevant later.

Table 3.1 : Origins of seed used in germination experiments

Species	Reference Code used in this Chapter	Origin (with grid reference) and date of collection
Ligusticum scoticum	L.s.5	Boarhills, Fife (NO 574151) 20.10.1975
	L.s.9	Fife Ness, Fife. (NO 637098) 24.10.1975.
	L.s.F76	Fife Ness, Fife. (NO 637098) 17.10.1976.
	L.s.Is	Port Ellen, Islay. (NR 372448) 1.9.1977.
	L.s.St.A	West sands, St. Andrews. (NO 503172) 18.10.1977.
	L.s.Crail	Caravan site, Crail, Fife. (NO 625079) 2.11.1977
	L.s.F77	Fife Ness, Fife (NO 637098) 2.11.1977.
Mertensia maritima	M.m	4th Barrier, Orkney (ND 481955) Aug. 1977
Crithmum maritimum	C.m.1	Great Orme, North Wales (SH 749840) 13.11.1976
	C.m.2	Merthyr-Mawr Warren, Glamorgan, S. Wales. (SS 7685) 12.10.1977
Limonium binervosum	L.b.	Tregantle, S. Devon (SX 388527) 19.8.1977
Glaucium flavum	G.f.	Botanic gardens, St. Andrews, 1675/72 originally from Prof. Tutin, Leicester as seed, B31/00N, Wolferton, Norfolk. 6.10.1977

MATERIALS AND METHODS

The seed used for the germination experiments was from a variety of sources as indicated in Table 3.1. Storage conditions varied considerably and are recorded for each individual experiment in the Results section. In this chapter the term 'seed' has been used throughout though single seeded fruits were used for Ligusticum scoticum, Mertensia maritima and Crithmum maritimum and single seeds for Limonium binervosum and Glaucium flavum, that is, the natural propagules for each species.

In all experiments Petri plates lined with Whatman No 3 filter paper were used, these being kept moist with distilled water. Each treatment consisted of two plates with 20 seeds in each unless stated otherwise; the few exceptions are noted individually in the Tables of the Results section. Sterilisation of seed with 1% sodium hypochlorite was tried to reduce visible fungus infection, but it was found to be of no advantage. Effective sterilisation was particularly difficult in the two Umbelliferous species, Ligusticum and Crithmum, where the membranous coating of the fruit was infected and attempts at sterilisation apparently stimulated growth and appearance of the fungus.

In all experiments the start was considered to be the time when the seeds were first moistened with distilled water. Plates were kept in temperature controlled cabinets accurate to $\pm 1^{\circ}C$ with no artificial lighting, and germination of seeds was recorded as having occurred when the tip of the radicle was clearly visible.

The percentage of seeds which had germinated by the time each experiment was terminated is referred to in the Tables of the Results section as 'Final % germination', and this value usually has increments of 2.5% which represents one seed in most experiments. An indication of the rate of germination is given by the number of days to the first germination, the day on which the last germination was observed (that is 'days to final % germination') and the number of days required for half of this final % germination. This last value is also used in the Figures to give an indication of the germination characteristics in a form similar to that used by Thompson (1970).

Three types of investigation were made into the response of seed germination to temperature.

1. Constant temperature

Seeds were kept at constant temperatures ranging from 5°C to 30°C (5° intervals), the length of each experiment being determined by the rate at which the seeds germinated at any particular temperature. If possible they were left until no further germination seemed likely, otherwise the longest time available was used. Seeds were kept in the dark but were exposed to available laboratory or day light when removed for examination at intervals which ranged from one to three days.

The Results section and Tables detail the seed sources and actual treatments used: these did not always include all possible temperatures in the range of 5 to 30°C, because of limitations on availability of cabinets and amounts of seed.

2. Alternating temperatures

Temperatures of 2,6,10,14,18,22 and 26°C were used and each treatment corresponded to seed kept alternately at one temperature (say x) for an 8 hour 'day' (9 am to 5 pm) and transferred to a different temperature (say y) which was lower except in two cases, (2/6 and 2/26) for a 16 hour 'night' (5 pm to 9 am). Each treatment is referred to in the Results section in the form x/y, and this rigorous daily alternation continued for the 50 days of the experiment, with a less rigorous extension for Crithmum. Treatments were all kept in the dark except for transfer and examination as in 1 above.

Details of the actual treatments are given in the Results section. Sufficient seed was available for Ligusticum and Crithmum only, for this experiment.

3. Temperature and Light interaction

This experiment considered the effect on germination at 15°C of an initial cold period at 5°C after moistening of the seed, with the additional variable light or dark conditions. Periods of 7 or 14 days at 5°C were used before transfer to a 15°C constant temperature cabinet for the remainder of the experiment. Controls were kept at 15°C constant for the full length of the experiment.

The 'light' treatments were exposed only to available laboratory lighting when examined. It was decided not to employ a continuous light source since it is well known that most light dependent physiological processes need only a very short exposure to light to initiate a response (see for example Wareing and Phillips, 1970, pp.240-241). The parallel 'dark' experiment, run simultaneously, excluded all light by keeping the Petri plates in light-tight, non-airtight boxes in the temperature cabinets, and by examining only in a darkroom using a green safelight.

Seed of Ligusticum, Crithmum and Glaucium was used for this experiment, and the full details are given in Table 3.8 in the Results section.

RESULTS AND DISCUSSION

1. Constant temperatures

The five species studied had different responses of germination to the temperatures in the range studied, (Tables 3.2 to 3.6).

Of the two northern species, considered initially irrespective of prior storage conditions, Ligusticum scoticum (Tables 3.2) has its most rapid and usually highest percentages of germination at a constant temperature of 20°C for most of the seed samples used. The day of first germination at 20°C was generally between 11 and 18 days and the percentages attained were between 47.5% and 100% except in the case of L.s.Is fresh seed (Table 3.2e) where only 7.5% germinated (but see below). By comparison the percentages of germination at 25°C are very much lower than at 20°C and are down to 0 or 2.5% in most experiments; the two exceptions are L.s.St.A and L.s.F76 with 60% and 45% germination respectively (Tables 3.2g and i). These differences in response of seed from different origins probably reflect some of the ecotypic variation in seed samples. This variation is again apparent when germination at 5°C is considered, percentages obtained being between 2.5% and 95% though in one experiment some germination was still occurring up to the end of the experiment at 155 days (Table 3.2a). Many of the experiments for Ligusticum which were shorter than this showed germination at 5°C up to the time when the experiment had to be terminated, and it is possible that further germination might have occurred in some cases if the experiment had been allowed to continue. The long period at 5°C could be equivalent to natural overwintering, with germination taking place in 'spring'. In these constant temperature experiments, seed kept at 10° and 15°C had very low percentages of germination, never exceeding 12.5% at either temperature for all seed samples except L.s.Is (Tables 3.2e and f). This seed differs in its germination characteristics from the other Ligusticum seed used, but it should be noted that this is the only seed which originated on the west coast of Scotland; all the other samples were from Fife (Table 3.1).

For all Ligusticum seed samples (except L.s.Is, Table 3.2e) the final percentage germination is higher at 5° and 20°C than at 10° and 15°C (and usually 25°C), and this general pattern is illustrated in Fig 3.1 for three examples. The time taken at each temperature to achieve half of the final observed percentage germination is also recorded in the Tables and is plotted for the three examples in Fig 3.1. It is seen that this time decreases with increasing temperature, up to 20°C, (but when no germination occurred it was not possible to include such a figure).

The other northern species, Mertensia maritima, had low final percentages of germination, never greater than 20% (Tables 3.3). No trends are apparent for this species from the available data though temperatures of 10° or 15°C and above seem to yield slightly higher percentages generally than 5°C. One of these results is represented graphically in Fig 3.2. Again as for Ligusticum higher temperatures reduce the number of days to reach half the final percentage of germination. Here it is relevant to note that Scott (1963a) only found germination percentages of about 20% at room temperature (mean 17°C) after a variety of dry storage conditions, as in this experiment; however, after cold (2°C) and wet storage for 33 days, 90% germination was attained in 10 to 12 days at room temperature.

The length of storage of seed and conditions of storage influence germination percentages of both northern species under constant temperature conditions to some extent, though results are variable. In some cases germination improves with increasing time of storage as shown by L.s.Is seed (Tables 3.2e and f) and Mertensia seed (Tables 3.3a and b), however the opposite effect, namely reduction in germination with storage, is found for L.s.9 seed (Tables 3.2a, b and c). Further experiments with more closely controlled storage times and temperatures would be needed to investigate this effect.

Of the southern species studied, Crithmum maritimum has much higher final percentages of germination at low temperatures than at high ones, (Tables 3.4). Germination is high, 30% to 70%, at 5°, 10°

and 15°C and only occurs at 20° and 25°C after storage for one year. Fig 3.3a shows this effect and also the decrease in time to half final percent germination from 5° to 15°C and the subsequent increase up to 25°C. There was no germination at 30°C. These results confirm those of Okusanya (1977) except that he found no germination at 5°C constant; however, his experiments lasted for only 7 weeks (49 days) which, in the light of present work, was barely long enough for germination at this temperature.

Glaucium flavum shows the same effect as Crithmum of maximum germination at the lower temperatures studied, however, the percentages attained were much lower, the greatest being 25% at 5°C after storage for 15 weeks (Table 3.5b). The low percentages of germination of Glaucium have been commented on by Scott (1963b) who found that maximum germination was obtained by planting the seed on sand and leaving it outside for the winter, and that germination improved after the seed has been stored for three years. Fig 3.3b for Glaucium shows this low temperature preference and also the decreasing time to half final percentage germination with increasing temperature for the three temperatures where germination occurred.

Limonium binervosum gives consistently high percentages of germination (87.5 to 97.5%) at constant temperatures of 10° to 25°C with a slight reduction to 69% at 30°C. Germination was very rapid for this species and was complete by day 15 of the 80 day experiment (Table 3.6). Since the original sample was possibly not fully mature when collected, it was unfortunate that not enough seed was available to test the effect of storage on germination of Limonium. Once again there is a decrease in time to half final percentage germination with increasing temperature (Fig 3.3c) though the differences are not so great as for the other species studied since germination was completed so much faster for Limonium.

While constant temperatures are almost never encountered by a plant in natural conditions in Britain (but see alternating temperature experiment below), these experiments do give an indication of the slight 'preference' for higher temperatures by the northern species, and the

definite 'preference' for lower temperatures by the southern species for the germination of their seed. These observations are in accordance with the generalisations made on temperature and seed germination by Billings and Mooney (1968) for northern species and by Thompson (1970) for southern, specifically Mediterranean, species.

Table 3.2 : Ligusticum scoticum. Germination at constant temperature.

Table 3.2a : L.s.9, stored cold (0°C) and dry for 3 weeks. Experiment length 155 days.

Experimental temp. °C	5°(i)	10°	20°	25°	30°*
Final % germination	87.5	7.5	77.5	0	0
1st germination day	66	26	13	-	-
Days to ½ final %	133	27	21	-	-
Days to final %	155	37	37	-	-

*50 days only

Table 3.2b : L.s.9, stored cold (0°C) and dry for 5 weeks. Experiment length 132 days.

Experimental temp. °C	5°	10°	20°	25°	30°**
Final % germination	90.0	5.0	52.5	0	0
1st germination day	49	13	13	-	-
Days to ½ final %	109	13	18	-	-
Days to final %	123	30	40	-	-

**40 days only

Table 3.2c : L.s.9, stored cold (0°C) and dry for 26 months. Experiment length 120 days.

Experimental temp. °C	5°	10°	15°	20°	25°
Final germination %	12.5	0	0	65.0	2.5
1st germination day	98	-	-	14	57
Days to ½ final %	103	-	-	20	-
Days to final %	106	-	-	48	57

(i) Germination still occurring at 5°C up to end of experiment. Further germination can not be ruled out.

\- Value not applicable.

Table 3.2 (continued) : Ligusticum scoticum. Germination at constant temperature.

Table 3.2d : L.s.5, stored cold (0°C) and dry for 17 weeks. Experiment length 68 days.

Experimental temp. °C	5°(i)	10°	15°	20°	25°
Final % germination	95.0	0	7.5	82.5	0
1st germination day	44	-	21	13	-
Days to ½ final %	48	-	24	20	-
Days to final %	68	-	26	42	-

Table 3.2e : L.s.Is, used fresh. Experiment length 80 days.

Experimental temp. °C	5°(i)	10°	15°	20°	25°	30°
Final % germination	2.5	0	15.0	7.5	0	0
1st germination day	77	-	28	18	-	-
Days to ½ final %	-	-	38	28	-	-
Days to final %	77	-	66	29	-	-

Table 3.2f : L.s.Is, stored dry at laboratory temperature (mean 17°C) for 14 weeks, then cold (0°C) and dry for 6 weeks. Experiment length 120 days.

Experimental temp. °C	5°(i)	10°	15°	20°	25°
Final % germination	55.0	12.5	37.5	47.5	0
1st germination day	76	67	32	32	-
Days to ½ final %	91	68	49	41	-
Days to final %	119	109	76	67	-

(i) Germination still occurring at 5°C up to end of experiment. Further germination can not be ruled out.

\- Value not applicable.

Table 3.2 (continued) : Ligusticum scoticum. Germination at constant temperature.

Table 3.2g : L.s.St.A, stored dry at laboratory temperature (mean 17°C) for 8 weeks then cold (0°C) and dry for 6 weeks. Experiment length 120 days.

Experimental temp. °C	5°	10°	15°	20°	25°
Final % germination	27.5	0	5.0	100.0	60.0
1st germination day	81	-	21	11	12
Days to ½ final %	91	-	21	12	23
Days to final %	111	-	29	18	48

Table 3.2h : L.s.Crail, stored dry at laboratory temperature (mean 17°C) for 6 weeks then cold (0°C) and dry for 6 weeks. Experiment length 120 days.

Experimental temp. °C	5°	10°	15°	20°	25°
Final % germination	17.5	2.5	0	97.5	2.5
1st germination day	81	25	-	12	32
Days to ½ final %	89	-	-	17	-
Days to final %	106	25	-	29	32

Table 3.2i : L.s.F76, stored cold (0°C) and dry for 15 months. Experiment length 120 days.

Experimental temp. °C	5°(i)	10°	15°	20°	25°
Final % germination	25.0	0	12.5	92.5	45.0
1st germination day	76	-	16	11	15
Days to ½ final %	95	-	20	14	20
Days to final %	119	-	22	33	34

(i) Germination still occurring at 5°C up to end of experiment. Further germination can not be ruled out.

\- Value not applicable

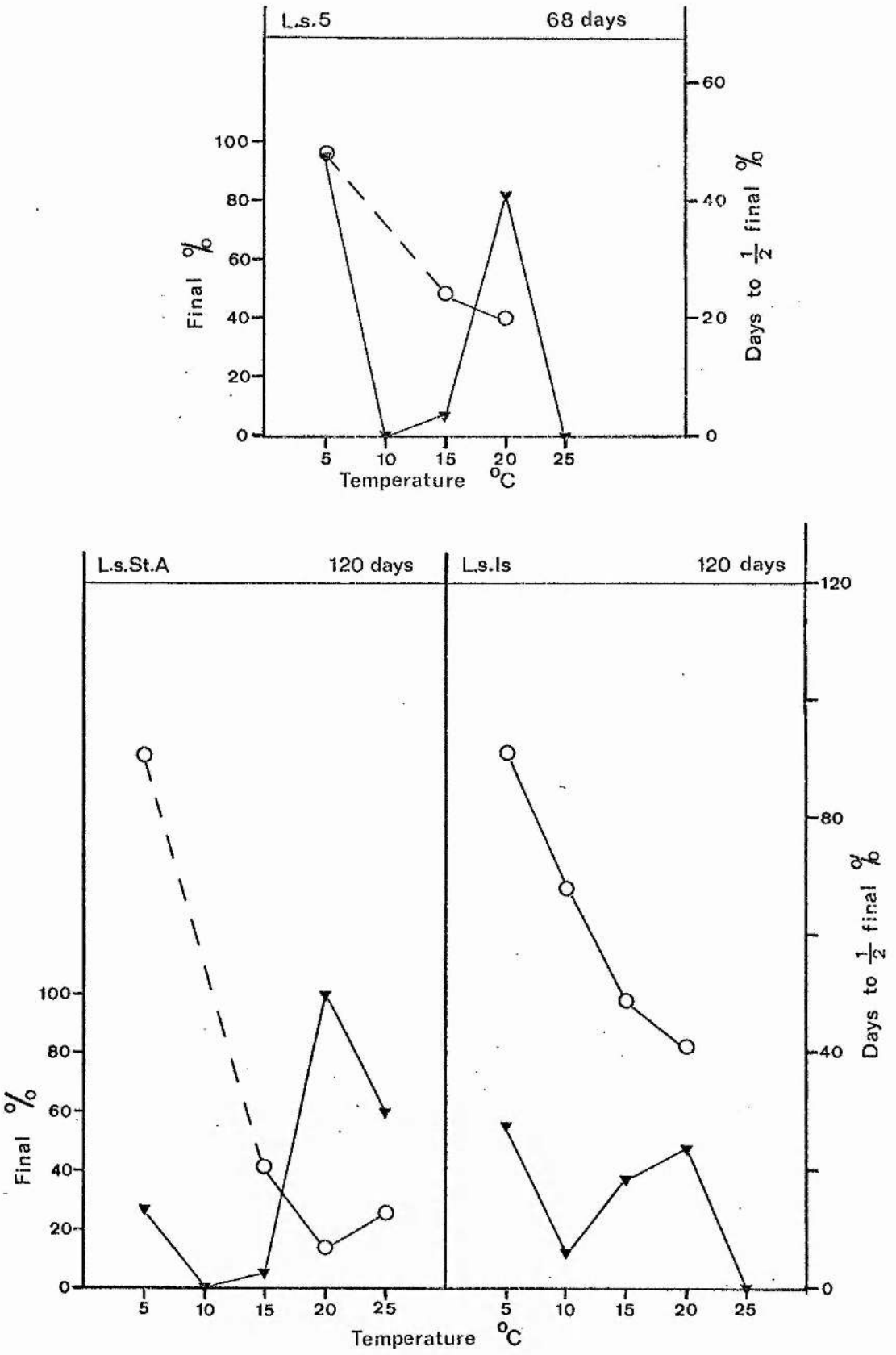


Fig 3.1: Ligusticum scoticum, germination at constant temperature.
Final % germination ▼
Days to ½ final % germination ○
Where no germination occurred there is no value for 'days to ½ final %'.
Length of each experiment indicated.
Data from Tables 3.2d, g and f.

Table 3.3 : Mertensia maritima Germination at constant temperature.

Table 3.3a : M.m., stored dry at laboratory temperature (mean 17°C) for 10 weeks.
Experiment length 80 days.

Experimental temp. °C	5°	10°	15°	20°	25°	30°
Final % germination	2.5	0	7.5	7.5	2.5	7.5
1st germination day	42	-	7	7	16	9
Days to ½ final %	-	-	12	27	-	17
Days to final %	42	-	48	30	16	48

Table 3.3b : M.m., stored dry at laboratory temperature (mean 17°C) for 17 weeks, then cold (0°C) and dry for 6 weeks.
Experiment length 120 days. (1x20 seeds in each treatment only.)

Experimental temp. °C	5°	10°	15°	20°	25°	30°
Final % germination	5.0	20.0	0	10.0	15.0	0
1st germination day	76	11	-	11	6	-
Days to ½ final %	-	18	-	11	6	-
Days to final %	76	98	-	16	11	-

- Value not applicable.

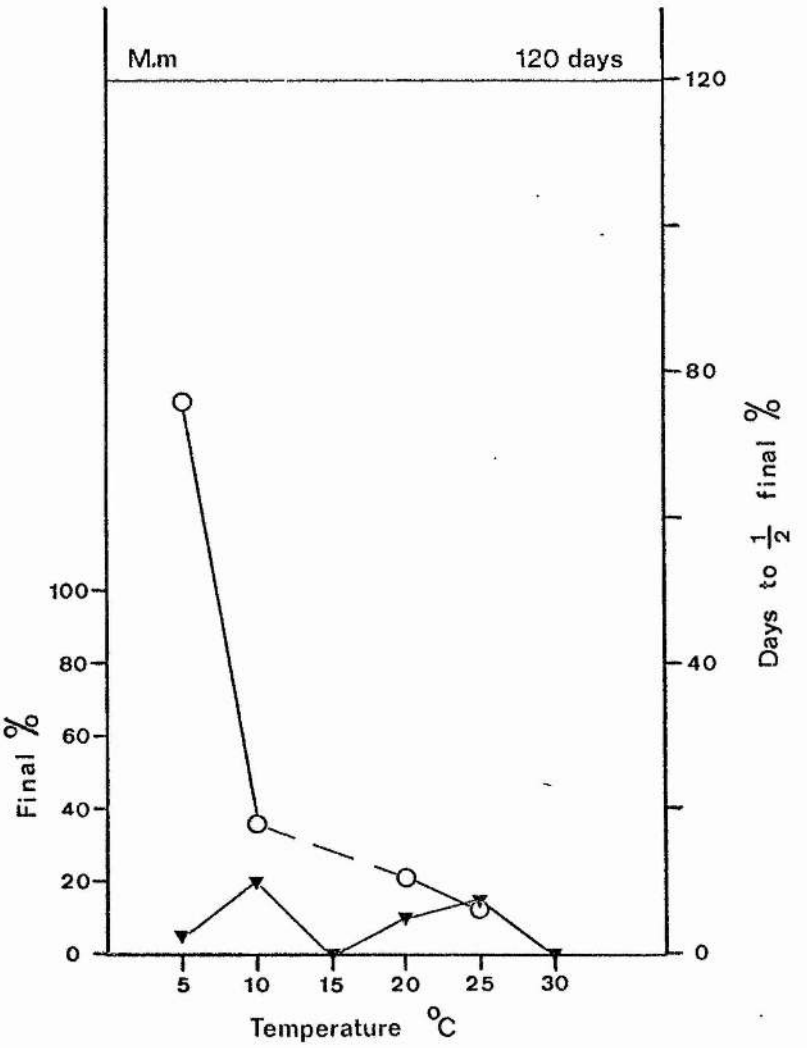


Fig 3.2: Mertensia maritima, germination at constant temperature.
Final % germination ▼
Days to ½ final % germination O
Where no germination occurred there is no value for 'days to ½ final %'.
Length of experiment 120 days.
Data from Table 3.3.

Table 3.4 : Crithmum maritimum. Germination at constant temperature.

Table 3.4a : C.m.1, stored dry at laboratory temperature (mean 17°) for 8 weeks.
Experiment length 85 days.

Experimental temp. °C	5°(i)	10°	15°	20°	25°	30°
Final % germination	52.5	50.0	30.0	0	0	0
1st germination day	64	25	20	-	-	-
Days to ½ final %	67	47	28	-	-	-
Days to final %	82	81	77	-	-	-

Table 3.4b : C.m.1, stored dry at laboratory temperature (mean 17°C) for 12 months.
Experiment length 80 days.

Experimental temp. °C	5°(i)	10°	15°	20°	25°	30°
Final % germination	55.0	67.5	50.0	7.5	5.0	0
1st germination day	43	20	23	28	69	-
Days to ½ final %	61	29	27	62	69	-
Days to final %	80	63	35	72	72	-

(i) Germination still occurring at 5°C up to end of experiment. Further germination can not be ruled out.

\- Value not applicable.

Table 3.5 : Glaucium flavum. Germination at constant temperature.

Table 3.5a : G.f., stored dry at laboratory temperature (mean 17°C) for 4 weeks.
Experiment length 80 days.

Experimental temp. °C	5°*	10°*	15°	20°	25°	30°
Final % germination	0	5.0	0	0	0	0
1st germination day	-	44	-	-	-	-
Days to ½ final %	-	44	-	-	-	-
Days to final %	-	49	-	-	-	-

* - a few seeds showed a split testa at the end of the experiment after treatment at 5 and 10°C but in no case did the radicle emerge from these split seeds.

Table 3.5b : G.f, stored dry at laboratory temperature (mean 17°C) for 9 weeks, then cold (0°C) and dry for 6 weeks.
Experiment length 120 days.

Experimental temp. °C	5°(i)	10°	15°	20°	25°
Final % germination	25.0	15.0	12.5	0	0
1st germination day	116	70	21	-	-
Days to ½ final %	118	94	24	-	-
Days to final %	120	100	36	-	-

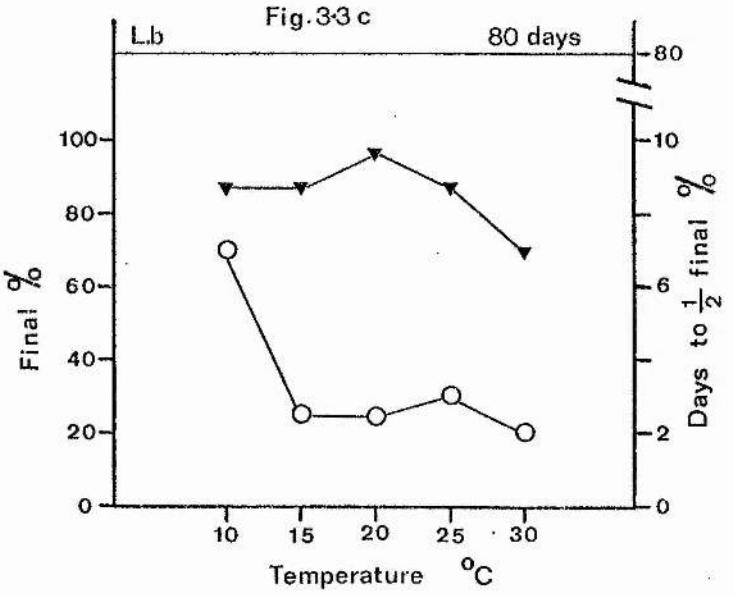
(i) Germination still occurring at 5°C up to end of experiment. Further germination can not be ruled out.

\- Value not applicable.

Table 3.6 : Limonium binervosum. Germination at constant temperature.

Table 3.6 : L.b, stored dry at laboratory temperature (mean 17°C) for 10 weeks. Doubtfully ripe when collected. Experiment length 80 days. 30° treatment only 13 seeds available, all other treatments 2 x 20 seeds.

Experimental temp. °C	10°	15°	20°	25°	30°
Final % germination	87.5	87.5	97.5	87.5	69.0
1st germination day	6	2	2	2	2
Days to ½ final %	7	2-3	2-3	3	2
Days to final %	9	6	3½	15	6



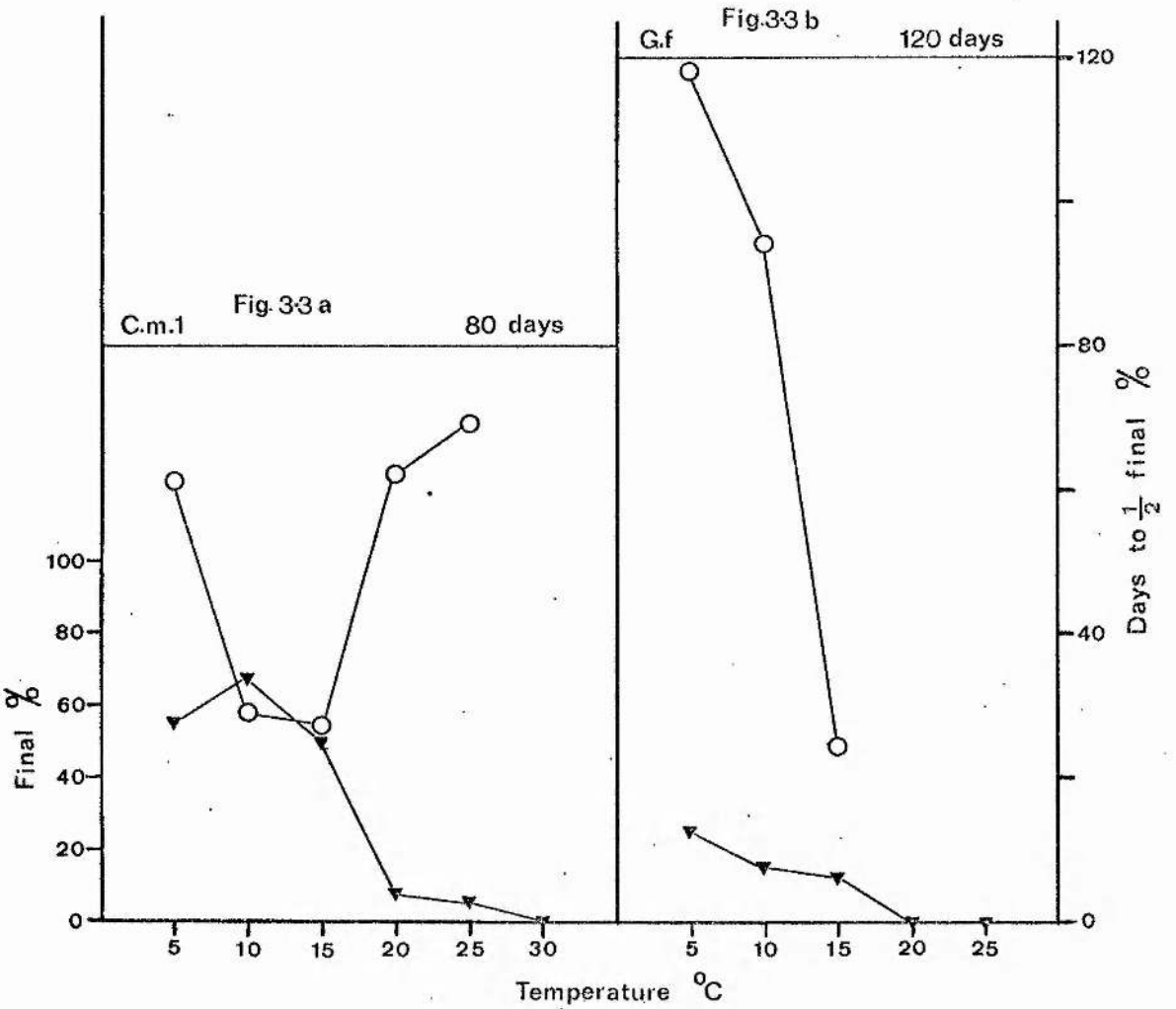


Fig 3.3: a, Crithmum maritimum(Table 3.4b); b, Glaucium flavum (Table 3.5b); c, Limonium binervosum (Table 3.6); germination at constant temperature.
Final % germination ▼
Days to ½ final % germination O
Where no germination occurred there is no value for 'days to ½ final %'.
Length of each experiment indicated.

2. Alternating Temperatures

The results of the alternating temperature experiments for Ligusticum scoticum and Crithmum maritimum are shown in Table 3.7. In these, as in the constant temperature experiments (Results 1 above), Ligusticum usually shows highest final percentage germination for higher temperature combinations or where one of the temperatures is around 22°C. As shown in Table 3.7 the highest germination (about 60%) results from a 'day' temperature of 18°, 22° or 26°C combined with a 'night' temperature of 10°C or more (but excluding 26/26 where only 2.5% germination occurred). The highest percentages of germination obtained are similar to those found in the constant temperature experiments; however, many different seed samples were used in those experiments and no direct comparison can be made with any reliability. An interesting point that emerges from this experiment is the low germination attained at temperatures close to 10° and 14°C with only small daily alternations of temperature. The results for 18/14 and 14/10 illustrate this point well, with 0 and 2.5% germination respectively, and these results help to confirm that the very low germination obtained at similar temperatures under constant conditions was a real effect (Results 1, above). At the lowest temperature combinations used it is possible that a longer time than the 50 days of the experiment was needed for any germination to take place, especially in view of the 60 to 100 days needed by seed of Ligusticum at 5°C in the constant temperature experiments.

Under these alternating temperature conditions, Crithmum, again as in the constant temperature experiments (Results 1, above), shows highest germination for lower temperature combinations, including at least one period at or below 14°C. Maximum germination percentages attained were 90% for 18/10 and 82.5% for 10/6, and these values are unequivocally greater than the maximum of 67.5% obtained in the constant temperature experiments (which used seed from the same batch). All treatments with a 10°C 'night' achieved germination of at least 50% after 50 days. When higher temperature combinations which are less

favourable for germination are reached there is a less abrupt reduction in percentages of germination obtained with Crithmum than occurs for Ligusticum when it is subjected to less favourable lower temperature conditions. Although from Table 3.7 it appears that treatments which have a 'night' temperature of 2°C do not attain very high germination for Crithmim, this is partly because the experiment only ran for 50 days with rigorous temperature alternation. It was continued with no alternation at weekends for a further 18 days, and this resulted in high percentages, from 30% to 72.5% for all treatments except 2/2 and 26/2. This extension confirmed that these lower temperatures are favourable for germination of Crithmum and further germination may still have occurred in a few of these treatments if they had been allowed longer.

For both Ligusticum and Crithmum, despite the different temperature ranges for optimum final percentage germination, the alternating treatments including higher temperatures have the shortest time to first germination, (when germination occurs at all) though there is no clear correlation between this and the percentage attained by the end of the experiment. For example at combinations with an 18°C night temperature, Ligusticum takes 10 or 11 days to first germination with 42.5 to 62.5% final germination and Crithmum takes 15 to 18 days with 27.5 to 32.5% final germination, while with a night temperature of 10°C the equivalent figures are 14 to 36 days and 2.5 to 32.5% for Ligusticum and 16 to 28 days and 50.0 to 90.0% for Crithmum

A comparison of the results in Table 3.7 with those of Tables 3.2 appears to show that the germination of Ligusticum is a few days faster overall under alternating temperature conditions than under constant ones, however the variation between results for different seed samples makes this difficult to quantify though the day of first germination is improved from 11 to 32 days at 20°C constant to only 10 or 11 days for alternating treatments with a 'night' temperature of 14°C or above. Though Crithmum has comparatively shorter times to first germination

under alternating temperatures, the overall time taken to reach the final percentage is similar to that for corresponding temperatures under constant conditions.

The differences in temperature requirements as reflected by these two alternating temperature experiments are summarised in Fig 3.4, which shows the higher optimum temperature combinations for good germination for Ligusticum and the lower ones for Crithmum.

The findings of Okusanya (1977) on the germination of Crithmum seed complement the present results by providing information on the response to different sea-water concentrations in addition to the various temperature treatments used.

3. Temperature and Light interaction

The results for these experiments (Table 3.8) show that the main effect of an initial cold period (at 5°C) is to increase the percentage germination obtained over that under constant temperature conditions at 15°C for Ligusticum and Glaucium in both light and dark, and for Crithmum in the dark only. The length of the initial period at 5°C also influenced the results, with greatest percentages of germination after the longer (14 day) initial cold period. This was most marked for the dark treated Ligusticum which had 5% germination at 15°C constant; 15% after 5°7 days than 15°const.; and 57.5% after 5°14 days then 15°const.. This effect, not necessarily in the dark, is really one of stratification or vernalisation of the seed which has been recognised for some time as one way of breaking dormancy and increasing germination (Wareing and Phillips, 1970).

When the results for germination of the seeds after light and dark treatments are compared (Table 3.8), it is seen that of the three species considered, only Crithmum has consistently greater germination in the light than in the dark. The differences however are small, and when a χ^2 test was applied (method from Campbell, 1967) only the 15°C constant

temperature experiment on this species showed a significant difference, at the 5% level, between light and dark treatments. Ligusticum does not show a consistent response to light and dark treatments; the 15°C constant and 5°7 days/15° const. treatments show greater germination in the light, and the remaining treatment 5°/14 days 15° const. has greater germination in the dark, though none of these differences is statistically significant. The germination achieved by Glaucium seed and the differences between treatments were so small that no conclusions can be drawn.

Table 3.7 : Ligusticum scoticum and Crithmum maritimum. Germination at alternating temperatures. Experiment length was 50 days of rigorous alternation for both species. Crithmum experiment continued for a further 18 days with no alternation at weekends, when the seeds were left at the lower 'night' temperature.

Seed used : L.s.F76, used fresh.
C.m.1, stored dry at laboratory temperature (mean 17°C) for 5½ months.

Where final percentage germination is recorded as 2.5% this represents one seed only.

- indicates value not applicable.

Table 3.7

Treatment Day/Night °C	Ligusticum scoticum				Crithmum maritimum				
	Final % germination	1st Day	Days to ½ final	Days to Final	Final % germination	1st Day	Days to ½ Final	Days to Final	Final % after 68 days
2/2 (const)	0	-	-	-	0	-	-	-	0
6/2	0	-	-	-	0	-	-	-	30.0
10/2	0	-	-	-	5.0	49	49	49	65.0
14/2	2.5	37	-	37	22.5	40	48	50	67.5
18/2	2.5	40	-	40	20.0	40	48	50	72.5
22/2	5.0	36	36	39	0	-	-	-	32.5
26/2	0	-	-	-	0	-	-	-	0
2/6	0	-	-	-	32.5	46	49	50	77.5
6/6 (const)					57.5	39	45	50	75.0
10/6	5.0	30	30	40	82.5	26	34	47	82.5
14/6	0	-	-	-	72.5	26	33	45	77.5
18/6	12.5	26	29	36	72.5	25	33	45	72.5
22/6	40.0	14	24	42	57.5	34	39	50	72.5
26/6	5.0	25	25	28	2.5	42	-	42	10.0
10/10 (const)	2.5	36	-	36	60.0	23	34	49	60.0
14/10	2.5	29	-	29	67.5	22	27	39	70.0
18/10	12.5	19	21	33	90.0	16	23	33	90.0
22/10	32.5	15	22	37	70.0	22	29	43	72.5
26/10	17.5	14	16	27	50.0	28	36	50	57.5
14/14 (const)					55.0	16	23	43	57.5
18/14	0	-	-	-	55.0	15	20	31	55.0
22/14	50.0	10	20	41	62.5	17	23	36	62.5
26/14	37.5	10	19	49	62.5	18	30	49	65.0
18/18 (const)	57.5	11	17	38	30.0	18	25	32	30.0
22/18	62.5	10	13	19	27.5	15	19	25	27.5
26/18	42.5	10	12	26	32.5	18	26	39	37.5
22/22 (const)					2.5	25	-	25	2.5
26/22	60.0	11	16	27	0	-	-	-	0
26/26 (const)	2.5	30	-	30	0	-	-	-	0
2/26	0	-	-	-	0	-	-	-	0

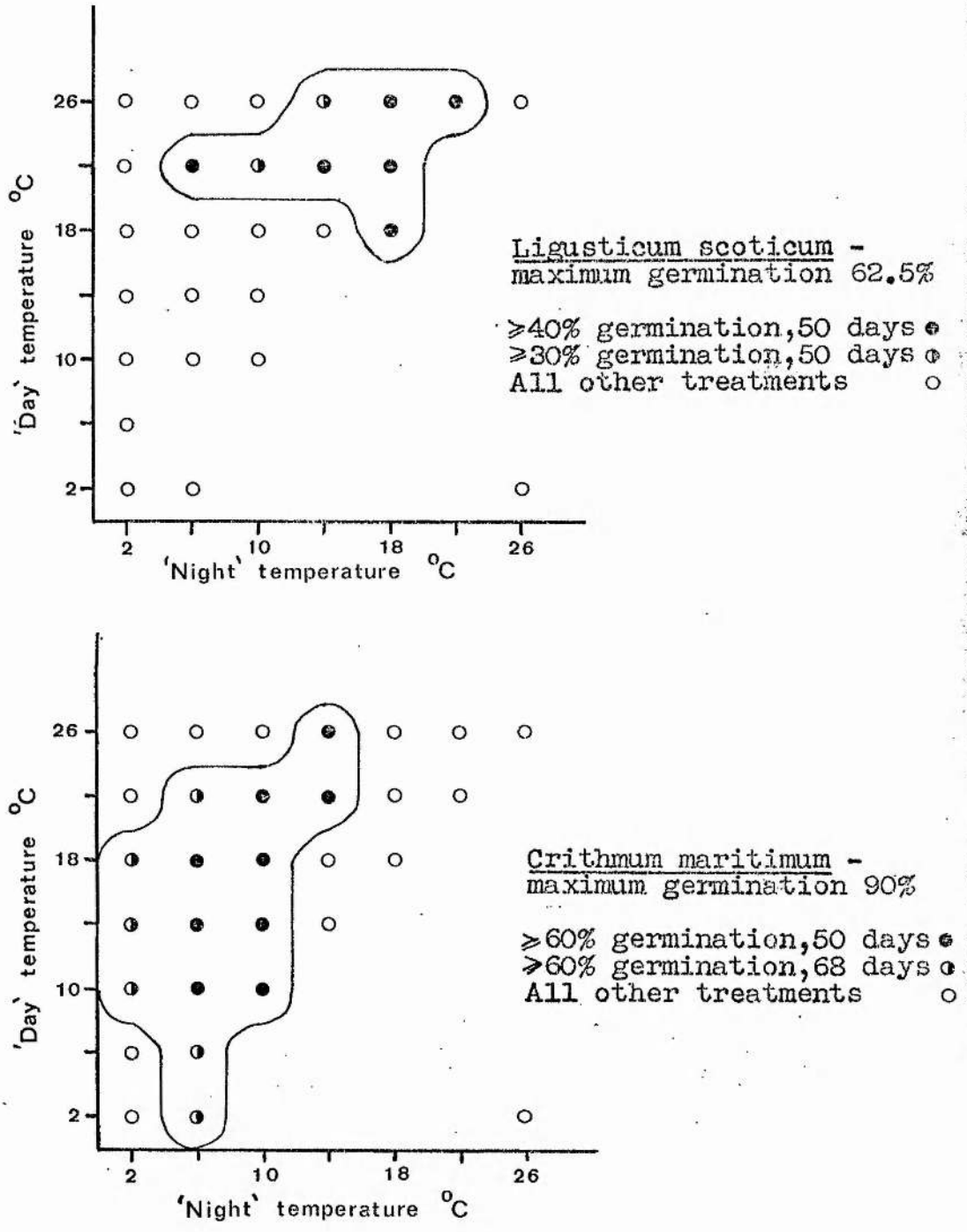


Fig 3.4: Ligusticum scoticum and Crithmum maritimum, germination at alternating temperatures.
Treatments which achieved ⅔ of maximum germination or more are indicated.
Individual values from Table 3.7.

Table 3.8 : Effect of temperature and light on the germination of Ligusticum scoticum, Crithmum maritimum and Glaucium flavum.

Seed used : L.s.F77, stored dry at laboratory temperature (mean 17°C) for 5½ months.
C.m.2, stored dry at laboratory temperature (mean 17°C) for 6 months.
G.f., stored dry at laboratory temperature (mean 17°C) for 9 weeks, then cold (0°C) and dry for 16 weeks.

Experiment length 70 days.

Treatment °C	Final % Germination	1st Day	Days to ½ Final %	Days to Final %	Final % Germination	1st Day	Days to ½ Final %	Days to Final %
	Light				Dark			
15° constant*								
L.s	15.0	18	29	48	5.0	30	-	30
C.m.	60.0	14	23	63	25.0	24	34	40
G.f.	0	-	-	-	0	-	-	-
5°/7days-15° const.**								
L.s.	27.5	18	28	37	15.0	20	20	40
C.m.	47.5	16	21	63	35.0	26	33	57
G.f.	0	-	-	-	0	-	-	-
5°/14days-15° const.**								
L.s.	45.0	26	29	63	57.5	22	27	44
C.m.	67.5	22	25	54	60.0	22	26	57
G.f.	7.5	24	28	37	2.5	22	-	22

* -- 1x20 seeds each treatment.

** -- 2x20 seeds each treatment.

All 'days' measured from beginning of experiment, e.g. for 5°/7 days--15° const.; days 0 to 7 at 5°C then day 8 onwards at 15°C etc. .

- Value not applicable.

CONCLUSIONS

These experiments have yielded the temperature requirements for germination of the seed of the five species studied with varying degrees of reliability.

Both northern species show some preference for warmer conditions for germination when kept at constant temperatures; around 20°C for Ligusticum, and 10° or 15°C to 30°C for Mertensia. In addition, Ligusticum often shows ability to germinate well at 5°C after a longer period of time. Experiments carried out on Ligusticum alone again showed its preference for higher temperature combinations, including one temperature around 22°C, when subjected to alternating daily temperatures. The enhancing effect of an initial continuous period of cold on seed germination found for Ligusticum is as discussed earlier, equivalent to the vernalisation or stratification procedures used on commercially grown plants.

The southern Crithmum shows highest percentages of germination at lower temperatures, 5° to 15°C, in constant temperature conditions, and even higher percentages, also at lower temperature combinations, in the daily alternation experiments with one temperature of 14°C or less. An initial period of cold only appears to enhance germination in the dark. Despite very low percentages of germination, Glaucium showed optimum performance between 5° and 15°C, especially after storage. Limonium appeared to germinate well at all the temperatures used (10° to 30°C) though it must be remembered that the seed may have been immature.

The slightly faster germination of Ligusticum and possibly faster germination of Crithmum at alternating temperatures over the times taken at roughly comparable constant temperatures have no effect on the optimum temperatures for germination of these two species, mentioned above; high for Ligusticum and low for Crithmum. These alternating temperatures are similar to the type of temperature regime to which the seeds would be subjected under their natural conditions. The effects of light and dark are less clear, though Crithmum does

seem to germinate better when it receives some light.

Length of time of seed storage had some effect on the results obtained for all species, but a more rigorous investigation of this would be necessary before any further conclusions could be drawn. The effect of storage may basically be one of 'after-ripening' and be time dependent.

The differences in optimum temperatures of germination between the northern and southern species reflect in the main their ecological adaptations to the conditions which they encounter in their natural habitats and are in accordance with the observations of Billings and Mooney (1968) and Thompson (1970) discussed in the introduction to this chapter.

CHAPTER 4

THE EFFECT OF TEMPERATURE ON ROOT RESPIRATION

INTRODUCTION

Dahl (1951) suggested that summer temperature was probably the factor which was limiting on the distribution of a number of Scandinavian alpine and arctic species, but he had no experimental data to support this. The effect of temperature on the respiration rate of arctic plants had already been measured by Wager (1941) who found that there was a greater response to temperature in the winter than in the summer for these species, and that the winter rates were higher than those for temperate species. He also reviewed other work in this area and compared the available data, which he found in the main to be in agreement with his results. Since then, many further investigations have been carried out on the direct response of respiration to temperature for both alpine and arctic species. Some of these are discussed below.

Higgins and Spomer (1976) investigated the influence of soil temperature on root respiration and its relation to the alpine timber-line. They concluded that soil temperature was more important than air temperature, and that it affected the response and thus distribution of plants at or near the timber-line. In addition, Anderson and McNaughton (1973) examined the effect of low soil temperature on other physiological processes such as photosynthesis and transpiration, in a range of species, and found these two processes to be less affected by a soil temperature of $3^{o}C$ than was the whole plant growth.

The effect of altitude on respiration, which indirectly reflects that of temperature, has been studied by Mooney (1963) in work on Polygonum bistortoides which has a range of ecotypes with different inherent rhizome respiration rates. The carbohydrate reserves of this plant are dependent on the physiological ecotypes and the corresponding differences in respiration rate, which is low for coastal populations and higher for alpine and sub-alpine populations. Extended work of a

similar type has also been published by Mooney and Billings (1960, 1961, 1965).

Similar work by Stewart and Bannister (1974) on the 3 British Vaccinium species (discussed in Chapter 1, page 2) shows comparable altitudinal effects on respiration rates to those above.

While all these studies are of considerable physiological importance, their value would have been enhanced by concurrent measurements of relevant climatic variables. This was in fact attempted by Stewart and Bannister (1973) when relating carbohydrate status of Vaccinium to time of year and the prevailing light and temperature conditions.

The survival of a plant is also affected by desiccation and direct damage at high and low temperatures, and by freezing damage at low ones alone. Riedmüller-Scholm (1974, 1976) by subjecting arctic and tundra plants to very high and low temperatures (to +60°C and - 80°C) found that the plants had a high survival rate when in their dormant winter condition, and were most susceptible to damage by these extreme temperatures in spring and autumn when the main climatic temperature changes are occurring.

As pointed out in Chapter 2 (page 9), northern coastal species in Britain seem to be limited by summer temperatures in a way similar to the southern limitation of arctic and alpine plants in Scandinavia, (Dahl, 1951). It is reasonable for northern plants to be tolerant of low temperatures since they regularly experience them, whereas they are subjected to very high temperatures much less often. Conversely, southern species, coastal or otherwise, in Britain appear to be limited by winter or summer cold (discussed in Chapter 2) and possibly show a wider diversity in their mechanisms of temperature tolerance.

Whatever the total effect of temperature on survival and distribution of a plant, there is no doubt that the rate of respiration and its relationship to temperature will be of some importance. For all species the temperatures experienced during the winter will affect the rate of respiration and so the directly resulting loss of stored carbohydrate and general depletion of the reserves in the plant which will, in turn, have a direct influence on its performance, and perhaps survival, in

the following year. This will be of greatest importance in species, usually those of northern distribution, which lose their leaves in winter and thus have no means of replenishing their reserves of carbohydrate. Southern species may retain at least some leaves and are able to take advantage of suitable conditions for some photosynthetic assimilation of carbohydrate to offset respiratory losses.

The above considerations were the basis for the study of the effect of temperature on respiration described in this chapter. The whole plants were subjected to a range of different temperatures to try to assess the effect of this in terms of potential carbohydrate loss and so survival as the plants emerged from their winter condition. The roots, or underground stems, of the plants were used in their winter state since not all had any significant shoot material above ground in winter.

Initially only northern species were examined in this way, so when the study was extended to the southern species the same emergence from winter condition was investigated to enable direct comparison between the two types. This meant that differences in behaviour or respiration response which could have a direct effect on the plants' survival could also be compared.

Table 4.1 : Origins of mature plants used in respiration experiments.

Species	Reference Code used in this Chapter	Origin (with grid reference) and date of collection or receipt
Ligusticum scoticum	L.s.	Collected West sands, St.Andrews. GR(NO 503172). 14.2.1976
	L.s.	Collected Boarhills, Fife. (NO 574151). 22.2.1976
	L.s.	Collected Boarhills, Fife. (NO 574151). 9.3.1977
Mertensia maritima	M.m.1	Collected N end (Burray end) of no. 4 barrier, Orkney. (ND 481955). In danger from dumping lorries and tractors. Coll. E. Bullard. 27.1.1977.
	M.m.2	Collected 4th barrier Orkney (ND 481955). Coll.R.M.M.Crawford Aug. 1977
Crithmum maritimum	C.m.	Collected Great Orme, Gwynedd, North Wales. (SH 749840). 2.1.1977
Limonium binervosum	L.b.B.	From Royal Botanic Garden, Edinburgh. Ac.no.720213, donor Berlin , Dahlem. Received 9.6.1977.
	L.b.V.	From Royal Botanic Garden, Edinburgh. Ac.no.582053, donor Vacratot. Received 9.6.1977
	L.b.J.	Collected Tregantle, S. Devon. (SX 388527) Coll. J.E.Palin. 19.8.1977
Glaucium flavum	G.f.	From self seeded stock, Botanic Garden, St.Andrews. St.A.1657/72, from Leicester as seed B31/00N, originally from Wolferton, Norfolk. 6.10.1977

MATERIALS AND METHODS

As stated in the Introduction, studies were carried out on mature plants in their winter condition. The sources of the plants and the reference code used in this chapter are listed in Table 4.1. The plants of Ligusticum scoticum and Crithmum maritimum were collected between January and early March prior to the experiments. Mertensia maritima, M.m.1 was used immediately on receipt; M.m.2 and Limonium binervosum from all three sources were planted in sand or in a sand/peat mixture in the summer, and kept in an open cold frame, protected from excessive waterlogging and potential rotting, until required. Glaucium flavum was treated similarly on receipt in autumn.

The plants of Ligusticum, Crithmum and Glaucium were mature, but not old, and had roots with diameters of 10 to 20 mm. The underground stems of Mertensia had a diameter of approximately 5 mm, while the woody roots of Limonium were 5 mm and the long fibrous roots 1 mm in diameter.

The respiration experiments were carried out between the months of January and March when the plants were in their winter condition: they had either died back as for Ligusticum and Mertensia, or were in their most dormant state as for Crithmum, Limonium and Glaucium. These last three, the southern species, did not lose their leaves completely but showed no visible growth over the winter prior to the temperature pretreatments described below.

At the start of an experiment the whole plant was removed from its pot, or collected fresh, and excess soil or sand was gently removed. It was then put into a sealed, air-filled polythene bag, sufficiently large to minimise changes in atmosphere, with some moist paper tissues to maintain humidity, and placed in a temperature controlled cabinet accurate to $\pm 1^{\circ}C$ in the dark. Experiments lasted for 1 to 4 weeks and the temperatures used were 5, 10, 15, 20 and $25^{\circ}C$. These combinations of temperature and time are known as the pretreatment conditions and are

expressed in the form $5^{\circ}/14$ days, $10^{\circ}/14$ days etc., or $5^{\circ}/2$ weeks etc throughout.

The pretreatment temperatures chosen were intended to reflect a range of conditions to which the plants, both northern and southern, might be subjected in their natural habitats as they came out of their winter condition. Ideally a pretreatment at $0^{\circ}C$ would also have been used, but this was not practicable with the equipment available. Though the higher temperatures and longer times of pretreatment resulted in conditions which would not normally be encountered in Britain, they were included to give as complete a picture as possible of the plants' response to temperature. It was hoped that the range of pretreatments would be great enough to show any differences in response between the northern and southern plants. Comparisons between species were facilitated by the constant conditions of each pretreatment. The actual pretreatments used for each species are detailed in the Results section.

Respiration was measured for the roots or underground stems of each pretreated plant at a range of experimental temperatures which were usually from $5^{\circ}C$ to $30^{\circ}C$ at 5° intervals, though for some earlier experiments the upper limit was $25^{\circ}C$. Later in the study confirmatory 'detailed experiments' were carried out over experimental temperature ranges suitable to the plant under investigation at 2° or $3^{\circ}C$ intervals. The respiration rates were measured on a Gilson respirometer, the principles and operation of which are described at the end of this section (page 82).

After careful washing, the plant root material used for respiration measurements was evenly sliced into flat discs of 1-2 mm thickness, the thinnest practicable by hand slicing, for all plants except Limonium where the fibrous roots were cut into 2-3 cm long pieces. 2.0 ml of phosphate buffer, at pH 5.8 to minimise carbon dioxide solubility to an acceptable level, was measured into each standard Warburg/Gilson flask

and a sample of 0.5g to 1.0g of fresh root material was put into this. Three samples were used from each plant for respiration measurement in terms of oxygen uptake, and where root material was limiting then that available was divided approximately equally between three flasks. If total gas change was also measured then three further root samples were used from the same plant (when available). In all cases the aim was to have sufficient respiring material to yield volume changes large enough for precise measurement on the Respirometer.

Measurement of oxygen uptake was made from flasks which had 0.4 ml of 2M KOH in the centre well, with a filter paper wick to increase surface area, for absorption of carbon dioxide. The centre well was left empty in flasks used for measurement of total gas change, though this was not always measured. All flasks were then connected to the Respirometer, immersed in the water bath at the initial temperature (usually 5°C) and left to come to equilibrium for 1 hour. The system was then closed off from the atmosphere and volume readings were taken for all the flasks at 5 minute intervals for up to 1 hour; the actual time depended on the rate at which the material was respiring. After this time the system was reopened to the atmosphere and the thermostatic control of the water bath was adjusted to the next higher experimental temperature and left to equilibrate for 50 minutes before the next set of readings were taken in the same way; and so on for each subsequent set of readings. The flasks were shaken mechanically at a rate of 80 times per minute throughout. At the end of the experiment the plant material from each flask was removed and dried to constant weight at 95°C.

Calculations of the rates of respiration, expressed as QO_2 and QCO_2 (where applicable) were made in terms of of µl of gas per mg dry weight per hour ($\mu l.mgDW^{-1}.hr^{-1}$) after correction of all gas volumes to standard conditions of 25°C and 760 mmHg. QO_2 was calculated directly from uptake volumes and for each plant is expressed as the mean of the three

values obtained. QCO_2 was calculated indirectly from the corrected volume of total gas change by adding the volume of O_2 appropriate to the dry weight, as calculated from the mean QO_2 from the same plant. Measurement of total gas change and thus calculation of QCC_2 was discontinued in later experiments since the gas volumes measured were usually so small as to be subject to unacceptable error (see Gilson Respirometer description below). The volumes of gas used for QO_2 calculation were large enough in most cases to eliminate this error; the exceptions to this are noted in the Results section and were usually due to shortage of root material.

The mean respiration rate (as QO_2) was plotted against the experimental temperature for each pretreated plant of each species used, and these curves were used for initial comparisons between species and pretreatments.

Further information was then extracted from the data by transforming each set to an Arrhenius plot of $\log_{10}$Rate against $\frac{1}{\text{Temperature}}$ which enabled the direct calculation of the energy of activation, E_a, from the gradient of the resulting line or lines.

The Arrhenius equation $k = A\, e^{-E_a/RT}$ is transformed to the straight line form

$$\log_{10} k = \log_{10} A - \frac{1}{T} \times \frac{E_a}{2.303R}$$

where k = respiration rate (QO_2)

T = temperature in K

R is the gas constant (1.987 cal.$^{\circ}C^{-1}$.mol^{-1})

A is a constant ($\log_{10} A$ represents the intercept of the Arrhenius plot)

E_a is activation energy

The Results are divided into two sections: Section A records the rate of O_2 uptake (QO_2) v temperature data, and Section B the Arrhenius plot data.

Gilson Respirometer (see Williams and Wilson, 1975)

The Gilson respirometer is a constant pressure manometer system. The constant pressure is maintained in the Respirometer by employing a large reference flask on the opposite side of the manometers to the experimental flasks. The reference flask is filled with distilled water to such a level that the resulting volumes of water saturated air on each side of the manometers are equal, rendering pressure equal for a constant temperature. This removes the necessity for use of a thermo-barometer flask which the constant volume Warburg manometers need, though a control flask (with no plant material) was used in all these experiments to enable correction to be made for fluctuations in volumes obtained due to unavoidable variations in laboratory temperature.

The flasks are all immersed in a temperature controlled water bath. The changes in volume resulting from respiration are calculated by difference from values read off directly from a scale calibrated in μl, after equalising levels in the 2 sides of the manometer for all the flasks in order. Very small changes in volume, less than 10 $\mu l.hr^{-1}$, are subject to error which is due to slight leakage or diffusion of gases through the 'Tygon' tubing of the manometer connections (this shortcoming of the method is pointed out in the manufacturers handbook though no method for obviation is suggested).

The volume readings were all corrected, by the gas laws, for atmospheric pressure and temperature to a standard 760 mmHg and 25°C (STP). The formula employed for this is:

$$V_2 = \frac{V_1(Pb-Pw-3)T_2}{T_1 \times P_2}$$

where

V_2 = corrected volume in μl (STP)

V_1 = experimental gas volume in μl

Pb = barometric pressure in mmHg

Pw = SVP of water at T_1 in mmHg

3 is a correction for SVP of manometer fluid (kerosene, dyed red)

T_1 = experimental temperature in K

T_2 and P_2 refer to STP i.e. 298K and 760 mmHg

RESULTS

A: Basic Respiration Data.

Graphs were plotted of the rate of respiration of the roots with respect to experimental temperature after the various time and temperature pretreatments. Fig. 4.1 shows a characteristically shaped curve for each of the four main species studied, Ligusticum scoticum, Mertensia maritima, Crithmum maritimum and Limonium binervosum. From this, two points of interest emerge, firstly that both the northern species, Ligusticum and Mertensia, have respiration rates which are approximately twice as great as those for the two southern species, Crithmum and Limonium over the entire range of experimental temperatures considered. Secondly, the form of the graphs differs; those of the northern species approximate to straight lines while those for the southern species are of a distinctly curved (exponential type) form over the temperature range studied. This difference in graph form helps to account for the differences in the Arrhenius plots which are discussed in the next section.

There is variation in the magnitude of the response of respiration to increasing experimental temperature after different pretreatment temperatures, and this is shown individually for each species in Tables 4.2 to 4.8 for both 5° interval and 'detailed' experiments and summarised in Table 4.9 for pretreatment temperatures only. The times, of one to four weeks, for which the pretreatments lasted were of less significance than the temperatures of pretreatment, and little additional information was gained by grouping the summarised results in this way.

The high respiration rates of the northern species and low ones of the southern species are summarised in brief form in Table 4.9(a) overleaf, with figures taken from Table 4.9.

Table 4.9 (a) : Overall means of respiration rates, QO_2 ($\mu l.mg^{-1}.hr^{-1}$) at experimental temperatures of 5°C and 30°C for Ligusticum scoticum and Mertensia maritima (northern), and Crithmum maritimum and Limonium binervosum (southern). The limits are ±S.E. (standard error) with the number of values used for each mean in brackets.

Species	5°C	30°C	Tables containing individual results.
Ligusticum	0.22 ±0.01 (18)	1.15 ±0.07 (10)	4.2 and 4.3 (detailed)
Mertensia	0.31 ±0.07 (16)	1.36 ±0.18 (16)	4.4 and 4.8 (detailed)
Crithmum	0.12 ±0.005(20)	0.52 ±0.02 (20)	4.5 and 4.6 (detailed)
Limonium	0.15 ±0.03 (14)	0.61 ±0.06 (14)	4.7 and 4.8 (detailed)

In order to make the data for all four species more directly comparable the individual results for each pretreatment were 'standardised' by expressing the respiration rates as a ratio of the rate at 5°C for each experimental temperature of a pretreated sample. For example: for Ligusticum pretreated 10°/6 days, from Table 4.2, the respiration rate (QO_2) at 5°C experimental temperature is 0.225 $\mu l.mg^{-1}.hr^{-1}$.; at 10°C, 0.371; and at 15°C, 0.560, etc and the corresponding ratios are:

for 10°C $\frac{0.371}{0.225}$ = 1.65, and for 15°C $\frac{0.560}{0.225}$ = 2.49, etc.

When presented in this way (Figs. 4.2 to 4.5) differences in respiration rate attributable to variations in proportion of respiring to non-respiring tissue are reduced, and the ways in which the rates change can then be directly compared for the northern and southern species, irrespective of the actual numerical values of respiration rate.

Of the two northern species, Ligusticum shows no very clear pattern of response of respiration to the temperature of pretreatment even after standardisation (summarised in Fig. 4.2): after 1 week and 2 weeks ('77) the maximum response, that is, the greatest proportional respiration rate increase with experimental temperature, is found after pretreatment at 20°C; after 2 weeks ('76) the pattern is unclear, but after 3 weeks the trend is for a reduction in response with increasing temperature of pretreatment.

Mertensia, the other northern species, has variable rates of respiration (Table 4.4) which are possibly due to variations in the proportions of actively respiring and thickened tissue, and for this reason the standardisation of data (summarised in Fig 4.3) is particularly helpful for direct comparison of the results. In contrast to Ligusticum, Mertensia shows an overall decrease in response to experimental temperature increase with increasing pretreatment temperature, and this is most marked between the 5°C and 10°C pretreatments after 2 weeks ('78). This effect is a possible direct compensation by the plant against its respiration rate staying too high when subjected to higher pretreatment temperatures.

The respiration data for the southern species, Crithmum (Table 4.5) and Limonium (Table 4.7) are shown as plots in the above standardised form in Figs 4.4 and 4.5 respectively. For Crithmum, overall changes in response with experimental temperature are so relatively small between the different pretreatments that they cannot be said to show any significant pattern, and are probably best taken as constant for the range of pretreatment temperatures investigated in these experiments. For Limonium, as for Mertensia, the plant material available was somewhat variable and in a few cases it was not possible to use more than the minimum amount necessary for the accurate measurement of respiration rates. The standardised responses of L.b.V after 2 weeks (Fig 4.5) show a downward trend with increasing temperature of pretreatment, and the combined results for L.b.B and L.b.J after 2 weeks appear to show a similar trend, though these latter were among the most erratic

for any species or treatment owing to the very small quantities of root used. The 3 week results are from plants of all three origins (Table 4.1) and show no clear pattern.

Another point which emerges from the standardised data plots (Figs 4.2 to 4.5) is that both the Umbelliferous species, the northern Ligusticum and the southern Crithmum, have much less variation and no clear trends in response attributable to the different time and temperature pretreatments than do either the northern Mertensia or the southern Limonium, which both show distinct reductions in response to experimental temperature after being subjected to higher temperatures of pretreatment.

For Glaucium flavum only one plant survived to the beginning of the respiration experiment and this was subjected to a detailed temperature experiment with results shown in Table 4.8. It has thus not been possible to include this additional southern species in the above discussion. From the single series of results, the actual rates of respiration are very high even when compared with those for the two northern species. Further measurements would have to be made to confirm this apparently anomalous result and to suggest a reason if it turns out to be a real effect.

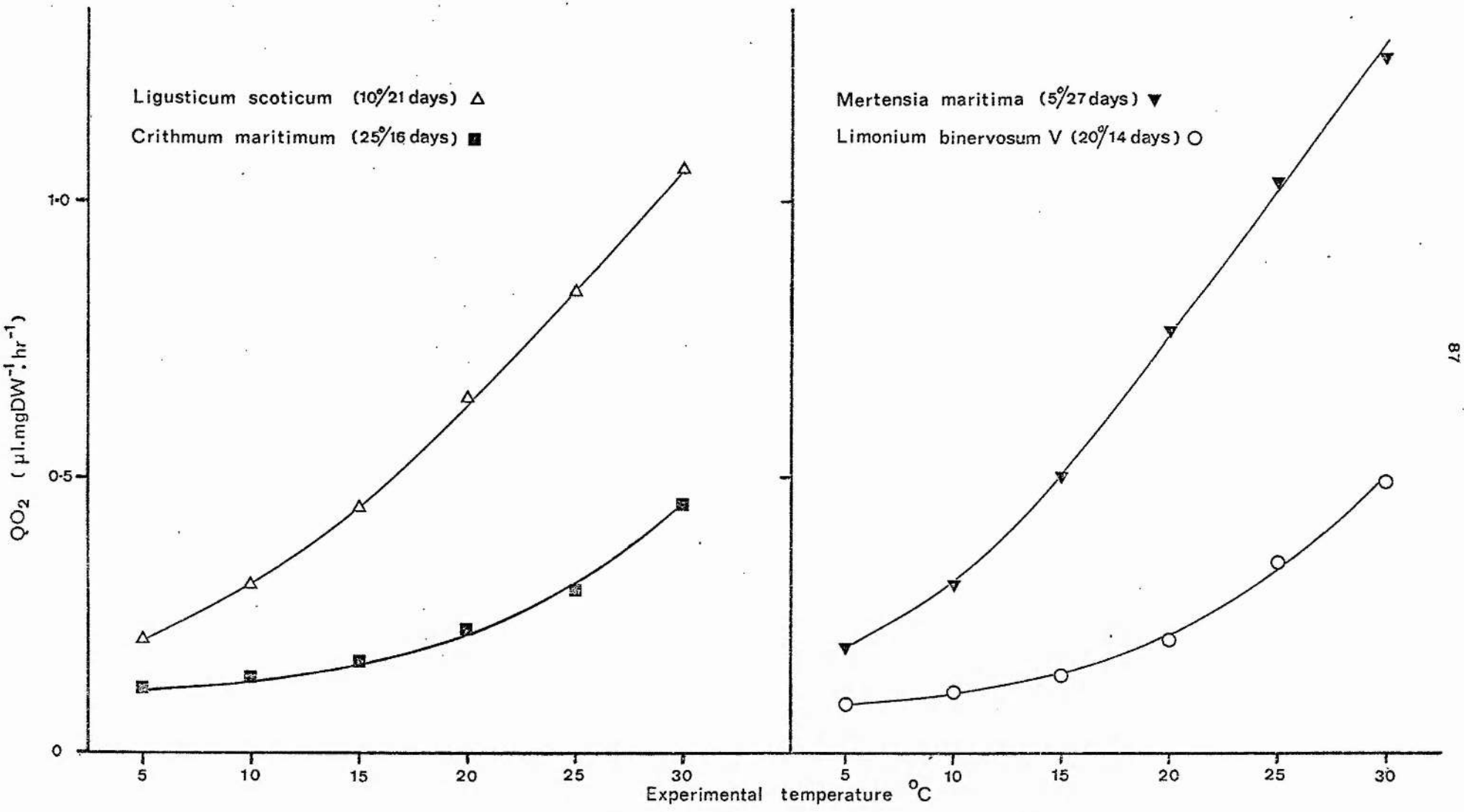


Fig 4.1: Respiration rate of roots at a range of experimental temperatures. Typical plots for two northern species, Ligusticum scoticum and Mertensia maritima, and two southern species, Crithmum maritimum and Limonium binervosum.

Table 4.2 : Respiration rate of root slices of Ligusticum scoticum expressed as Q_{O_2} ($\mu l O_2 . mgDW^{-1} . hr^{-1}$)

Pretreatment	Experimental Temperature (°C) 5°	10°	15°	20°	25°	30°
10°/ 6 days '76(1 week)	0.225	0.371	0.560	0.773	0.847	-
15°/ 6 days "	0.158	0.243	0.375	0.535	0.717	-
20°/ 7 days "	0.183	0.284	0.488	0.699	0.879	-
25°/ 7 days "	0.279	0.427	0.626	0.836	1.056	-
10°/15 days '76(2 weeks)	0.180	0.270	0.469	0.726	0.968	-
15°/15 days "	0.327	0.486	0.731	0.857	0.942	-
20°/16 days "	0.185	0.242	0.390	0.570	0.777	-
25°/16 days "	0.242	0.390	0.586	0.676	0.730	-
5°/14 days '77(2 weeks)	0.169	0.260	0.454	0.606	0.723	0.837
10°/15 days "	0.268	0.385	0.616	0.820	0.978	1.086
15°/15 days "	0.272	0.374	0.668	1.064	1.279	1.473
20°/16 days "	0.150	0.256	0.431	0.726	0.886	1.001
25°/16 days "	0.272	0.404	0.579	0.820	1.039	1.233
5°/20 days '77(3 weeks)	0.205	0.305	0.471	0.725	1.000	1.334
10°/21 days "	0.203	0.304	0.446	0.642	0.837	1.059
15°/21 days "	0.289	0.392	0.685	1.061	1.250	1.435
20°/22 days "	0.216	0.304	0.476	0.728	0.882	0.974
25°/22 days "	0.236	0.325	0.482	0.765	0.953	1.070

Notes: 1 - Each plant sample was subjected sequentially to the whole range of experimental temperatures, from the lowest to the highest, allowing 50 mins. for equilibration between each set of readings.

2 - Each value of Q_{O_2} is the mean of three measurements after correction to 25°C and 760 mmHg. The third decimal place is not usually significant but is included here since these results are later averaged for the compilation of Table 4.9.

Table 4.3 : Respiration rate of root slices of Ligusticum scoticum expressed as QO_2 ($\mu l\ O_2 \cdot mg\ DW^{-1} \cdot hr^{-1}$) for a detailed range of experimental temperatures at 2°C intervals.

	Experimental temperature (°C)										
	10°	12°	14°	16°	18°	20°	22°	24°	26°	28°	30°
5°/19 days (3 weeks)	0.372	0.454	0.558	0.663	0.765	0.865	0.966	1.066	1.152	1.196	1.335
15°/19 days "	0.436	0.513	0.638	0.787	0.915	1.002	1.139	1.255	1.339	1.401	1.519
10°/28 days (4 weeks)	0.268	0.362	0.471	0.529	0.604	0.671	0.756	0.813	0.886	0.949	1.035
20°/28 days (4 weeks)	0.313	0.380	0.470	0.572	0.652	0.682	0.774	0.854	0.937	1.012	1.147

Notes as for Table 4.2

Table 4.4 : Respiration rate of underground stem slices of Mertensia maritima expressed as QO_2 ($\mu l O_2 . mgDW^{-1} . hr^{-1}$.)

Pretreatment	Experimental Temperature (°C) 5°	10°	15°	20°	25°	30°
5°/15 days (2 weeks) '77	0.184	0.331	0.464	0.802	1.164	1.395
10°/16 days "	0.173	0.279	0.302	0.507	0.816	0.970
15°/16 days "	0.164	0.258	0.282	0.449	0.727	0.928
20°/17 days "	0.165	0.225	0.306	0.397	0.500	0.645
25°/17 days "	0.165	0.242	0.320	0.414	0.519	0.668
5°/27 days (4 weeks) '77	0.189	0.305	0.505	0.768	1.039	1.263
10°/28 days "	0.175	0.262	0.355	0.470	0.714	1.270
15°/28 days "	0.151	0.208	0.281	0.339	0.511	0.801
20°/29 days "	0.192	0.283	0.314	0.401	0.483	0.569
25°/29 days	0.219	0.320	0.332	0.481	0.578	0.745
5°/14 days (2 weeks) '78	0.182	0.346	0.619	0.897	1.262	1.441
ŧ10°/14 days "	0.770	0.610	1.123	1.209	1.473	1.757
ŧ15°/14 days "	0.838	1.209	1.382	1.885	2.208	3.068
ŧ20°/14 days "	0.913	1.044	1.476	1.966	1.897	2.313
5°/21 days (3 weeks) '78	0.222	0.303	0.645	1.079	1.414	1.662
10°/21 days "	0.325	0.507	0.795	1.413	2.001	2.215

ŧ - These results are less reliable because the amount of root material available only yielded Respirometer gas volumes of 10 $\mu l . hr^{-1}$ or less at the lower experimental temperatures.

Notes as for Table 4.2

Table 4.5 : Respiration rate of roots of Crithmum maritimum expressed as Q_{O_2} ($\mu l O_2 . mgDW^{-1} . hr^{-1}$.)

Pretreatment	Experimental temperature (°C)					
	5°	10°	15°	20°	25°	30°
5°/6 days (1 week)	0.062	0.105	0.170	0.282	0.429	-
10°/6 days "	0.130	0.147	0.208	0.345	0.477	-
15°/6 days "	0.129	0.152	0.217	0.359	0.498	-
20°/7 days "	0.161	0.210	0.288	0.452	0.599	-
25°/7 days "	0.097	0.123	0.168	0.272	0.398	-
5°/15 days (2 weeks)	0.117	0.149	0.222	0.332	0.450	0.561
10°/15 days "	0.119	0.141	0.195	0.272	0.391	0.480
15°/15 days "	0.126	0.153	0.213	0.287	0.393	0.494
20°/16 days "	0.155	0.173	0.214	0.303	0.396	0.554
25°/16 days "	0.118	0.132	0.165	0.222	0.291	0.452
5°/21 days (3 weeks)	0.126	0.152	0.218	0.311	0.397	0.456
10°/21 days "	0.109	0.159	0.202	0.306	0.425	0.549
15°/21 days "	0.116	0.148	0.184	0.268	0.375	0.479
20°/22 days "	0.120	0.154	0.213	0.276	0.374	0.459
25°/22 days "	0.105	0.133	0.176	0.254	0.356	0.444
5°/28 days (4 weeks)	0.168	0.202	0.294	0.378	0.539	0.674
10°/28 days "	0.158	0.186	0.237	0.290	0.391	0.587
15°/28 days "	0.130	0.163	0.220	0.285	0.396	0.557
20°/29 days "	0.144	0.174	0.207	0.286	0.407	0.553
25°/29 days "	0.114	0.130	0.166	0.228	0.330	0.473

Notes: as for Table 4.2

Table 4.6 : Respiration rate of roots of Crithmum maritimum expressed as QO_2 ($\mu l O_2 . mgDW^{-1} . hr.^{-1}$) for a detailed range of experimental temperatures at 2°C intervals.

	Experimental Temperature (°C)													
Pretreatment	3°	5°	7°	9°	11°	13°	15°	17°	19°	21°	23°	25°	27°	29°
5°/49 days (7 weeks)	0.120	0.123	0.125	0.145	0.184	0.204	0.259	0.316	-	-	-	-	-	-
5°/48 days (7 weeks)	-	-	-	-	-	-	0.219	0.242	0.327	0.349	0.453	0.525	0.624	0.784
15°/49 days (7 weeks)	0.117	0.112	0.114	0.123	0.147	0.162	0.207	0.257	-	-	-	-	-	-
15°/48 days (7 weeks)	-	-	-	-	-	-	0.210	0.206	0.246	0.265	0.326	0.374	0.456	0.649

Notes: as for Table 4.2

Table 4.7 : Respiration rate of root slices (or pieces, denoted by*) of Limonium binervosum expressed as QO_2 ($\mu l O_2 . mgDW^{-1} . hr^{-1}$.).

	Pretreatment		Experimental temperature (°C) 5°	10°	15°	20°	25°	30°
♣*	L.b.B 5°/14 days	(2 weeks)	0.081	0.152	0.313	0.292	0.424	0.541
♣*	L.b.B 10°/14 days	"	0.184	0.148	0.196	0.273	0.395	0.358
*	L.b.J 15°/14 days	"	0.364	0.472	0.463	0.604	0.649	0.834
♣*	L.b.J 20°/14 days	"	0.403	0.268	0.500	0.686	0.641	0.890
	L.b.V 5°/14 days	(2 weeks)	0.064	0.097	0.159	0.242	0.348	0.463
	L.b.V 10°/14 days	"	0.104	0.171	0.297	0.442	0.594	0.705
	L.b.V 15°/14 days	"	0.108	0.153	0.248	0.381	0.552	0.711
	L.b.V 20°/14 days	"	0.087	0.110	0.140	0.209	0.345	0.495
	L.b.V 25°/14 days	"	0.196	0.207	0.243	0.343	0.499	0.739
*	L.b.B 5°/21 days	(3 weeks)	0.078	0.126	0.158	0.216	0.278	0.317
*	L.b.B 10°/21 days	"	0.069	0.124	0.157	0.213	0.289	0.298
♣*	L.b.J 15°/21 days	"	0.197	0.295	0.378	0.366	0.724	0.939
♣*	L.b.J 20°/21 days	"	0.117	0.177	0.227	0.217	0.429	0.566
	L.b.V 25°/20 days	"	0.122	0.160	0.207	0.256	0.435	0.758

♣ - These results are less reliable because the amount of root material available only yielded Respirometer gas volumes of 10 $\mu l . hr^{-1}$ or less for the lower experimental temperatures.

Notes: as for Table 4.2

Table 4.8 : Respiration rate of roots of Mertensia maritima, Limonium binervosum and Glaucium flavum expressed as Q_{O_2} ($\mu l O_2 . mgDW^{-1} . hr.^{-1}$) for a detailed range of experimental temperatures at 3°C intervals.

	Experimental temperature (°C)									
Pretreatment	4°	7°	10°	13°	16°	19°	22°	25°	28°	31°
Mertensia maritima										
♣ 20°/17 days	0.687	0.653	0.818	0.987	1.360	1.401	1.734	1.901	2.153	2.329
Limonium binervosum										
L.b.V 5°/1 day	0.067	0.085	0.111	0.133	0.172	0.221	0.236	0.320	0.393	0.591
L.b.B 15°/17 days	0.107	0.114	0.151	0.191	0.265	0.289	0.346	0.400	0.542	0.752
Glaucium flavum										
5°/1 day	0.317	0.370	0.502	0.686	0.865	1.042	1.236	1.468	1.875	2.599

♣ - This result is less reliable because the amount of root material available only yielded Respirometer gas volumes of 10 $\mu l . hr^{-1}$ or less for the lower experimental temperatures.

Notes: as for Table 4.2.

Table 4.9 : Mean values of QO_2 ($\mu l O_2 . mgDW^{-1} . hr^{-1}$) for Ligusticum scoticum, Mertensia maritima, Crithmum maritimum and Limonium binervosum at 5°, 25° and 30°C experimental temperatures for each pretreatment temperature.

		Experimental Temperature °C					
Pretreatment		5°		25°		30°	
Ligusticum scoticum	5°	0.19	±0.02 (2)	0.86	±0.14 (2)	1.08	±0.25 (2)
	10°	0.22	±0.02 (4)	0.91	±0.04 (4)	1.07	±0.01 (2)
	15°	0.26	±0.04 (4)	1.05	±0.13 (4)	1.45	±0.02 (2)
	20°	0.18	±0.01 (4)	0.86	±0.03 (4)	0.99	±0.01 (2)
	25°	0.26	±0.01 (4)	0.94	±0.07 (4)	1.15	±0.08 (2)
All treats.		0.22	±0.01(18)	0.93	±0.04(18)	1.15	±0.07(10)
Mertensia maritima	5°	0.19	±0.01 (4)	1.22	±0.08 (4)	1.44	±0.08 (4)
	10°	0.36	±0.14 (4)	1.25	±0.30 (4)	1.55	±0.27 (4)
	15°	0.38	±0.23 (3)	1.15	±0.53 (3)	1.60	±0.73 (3)
	20°	0.42	±0.24 (3)	0.96	±0.47 (3)	1.18	±0.57 (3)
	25°	0.19	±0.03 (2)	0.55	±0.03 (2)	0.71	±0.04 (2)
All treats.		0.31	±0.07 (16)	1.08	±0.14(16)	1.36	±0.18(16)
Crithmum maritimum	5°	0.12	±0.02 (4)	0.45	±0.03 (4)	0.56	±0.06 (3)
	10°	0.13	±0.01 (4)	0.42	±0.02 (4)	0.54	±0.03 (3)
	15°	0.12	±0.003 (4)	0.41	±0.03 (4)	0.51	±0.02 (3)
	20°	0.14	±0.01 (4)	0.44	±0.05 (4)	0.52	±0.03 (3)
	25°	0.11	±0.005 (4)	0.34	±0.02 (4)	0.46	±0.01 (3)
All treats.		0.12	±0.005(20)	0.42	±0.02(20)	0.52	±0.02(15)
Limonium binervosum	5°	0.07	±0.005 (3)	0.35	±0.04 (3)	0.44	±0.07 (3)
	10°	0.12	±0.03 (3)	0.43	±0.09 (3)	0.45	±0.13 (3)
	15°	0.22	±0.07 (3)	0.64	±0.05 (3)	0.83	±0.07 (3)
	20°	0.20	±0.10 (3)	0.47	±0.09 (3)	0.65	±0.12 (3)
	25°	0.16	±0.04 (2)	0.47	±0.03 (2)	0.75	±0.01 (2)
All treats.		0.15	±0.03 (14)	0.47	±0.04(14)	0.61	±0.06(14)

Notes: 1 - The means for each pretreatment temperature include all pretreatment times for that temperature (Tables 4.2, 4.4, 4.5 and 4.7)

2 - Values given ± standard error (see Campbell, 1967). The number of values used for each mean is given in brackets.

3 - Values at 25°C experimental temperature are included since not all pretreatments were subjected to 30°C experimental temperature.

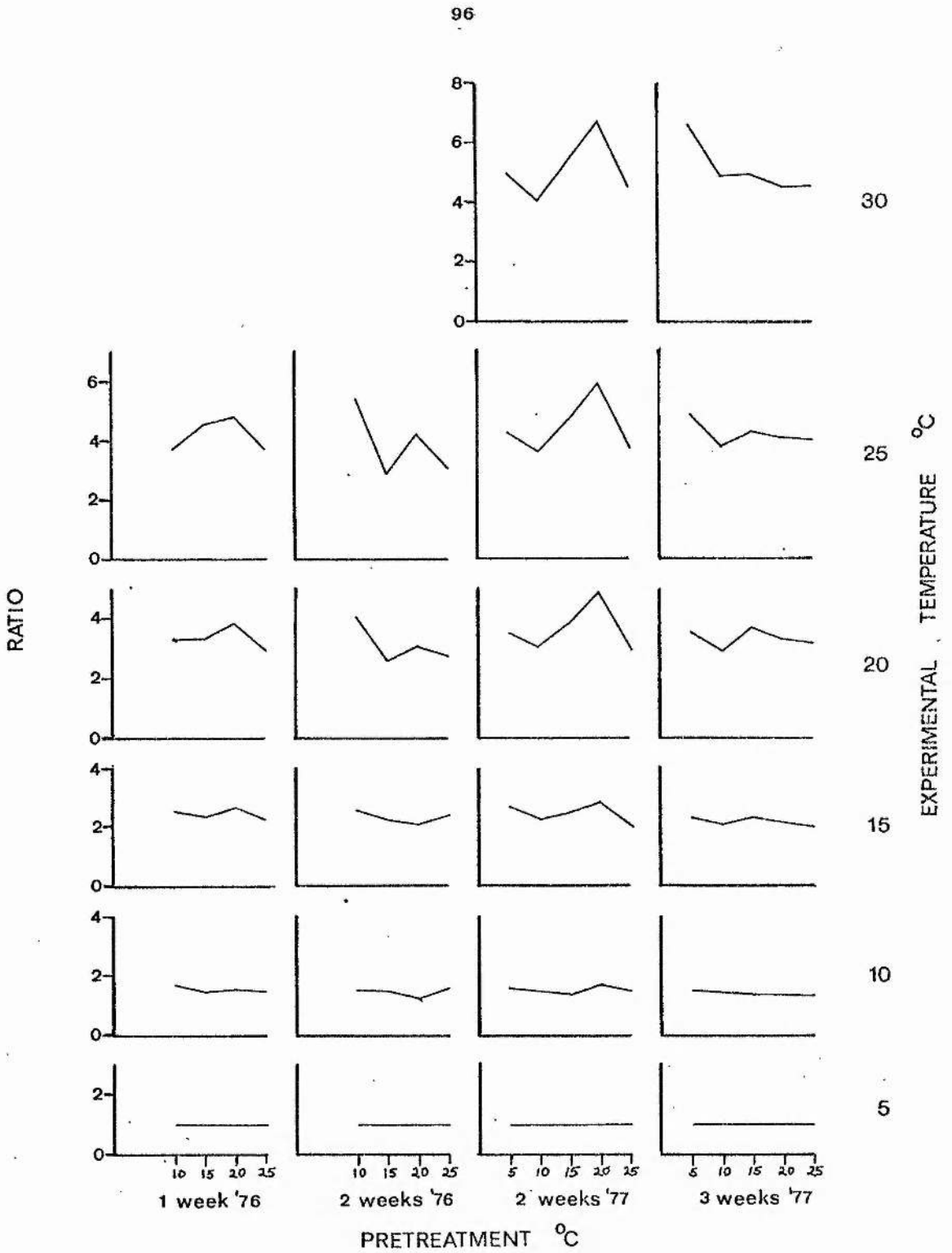


Fig 4.2: Ligusticum scoticum, standardised root respiration rates. Rates at each experimental temperature are expressed as a ratio of the rate at 5°C. Calculated from data of Table 4.2.

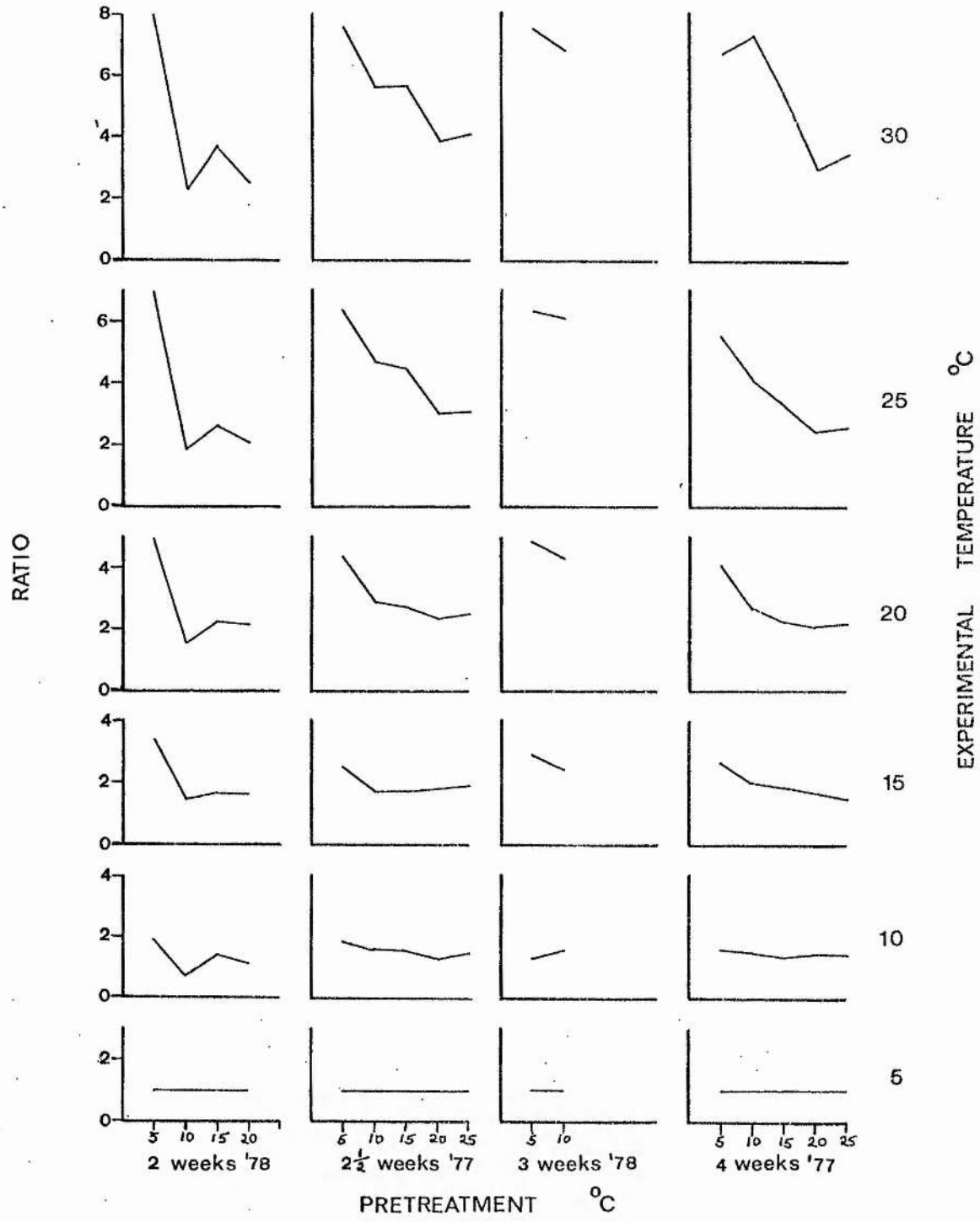


Fig 4.3: Mertensia maritima, standardised root respiration rates. Rates at each experimental temperature are expressed as a ratio of the rate at 5°C. Calculated from data of Table 4.4.

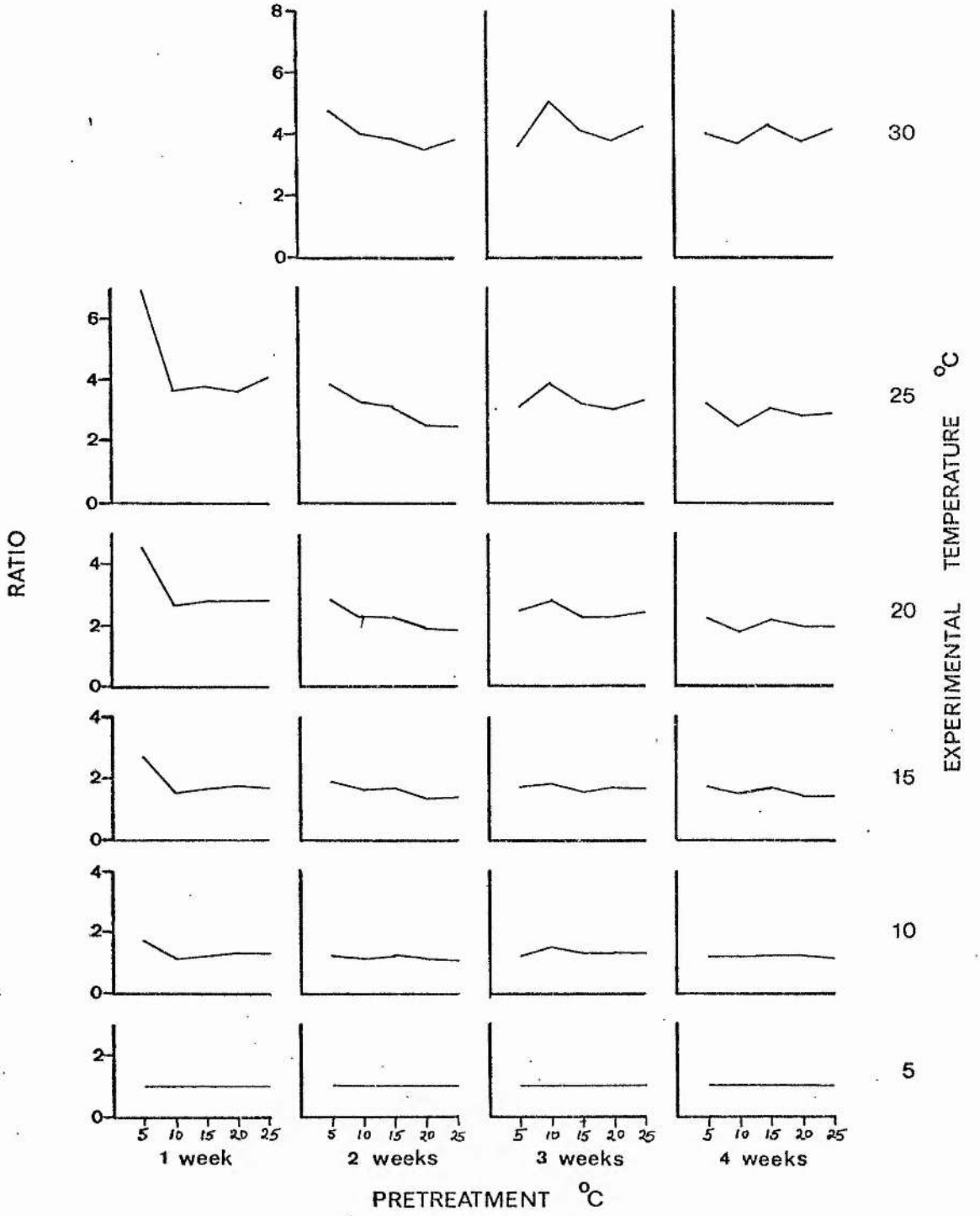


Fig 4.4: Crithmum maritimum, standardised root respiration rates. Rates at each experimental temperature are expressed as a ratio of the rate at 5°C. Calculated from data of Table 4.5.

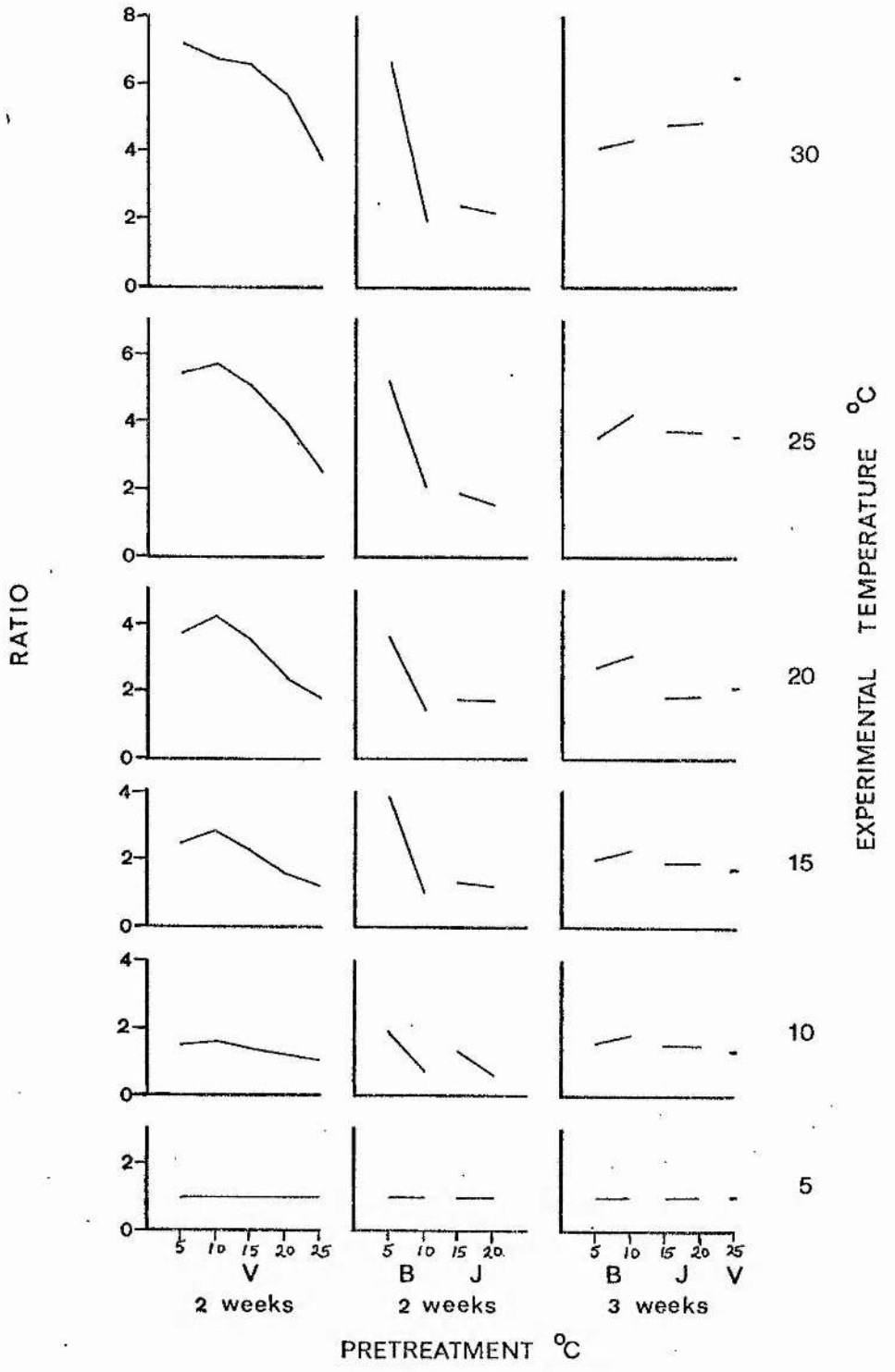


Fig 4.5: Limonium binervosum, standardised root respiration rates. Rates at each experimental temperature are expressed as a ratio of the rate at 5°C. Calculated from data of Table 4.7.

B. Transformed Respiration Data

As stated earlier, (page 81) the rate of respiration (QO_2) v. temperature graphs were all redrawn as Arrhenius plots of $\log_{10}$Rate against $\frac{1}{\text{Temperature}}$. In the 'ideal' or non-biological case of exponential increase of rate of reaction with temperature the Arrhenius plot shows a straight line, and the gradient of this line can then be used for direct calculation of the energy of activation (E_a) for the whole temperature range (as indicated on page 81). For biological data this 'ideal' condition is seldom reached, though it may appear to apply over certain limited ranges of temperature. The Arrhenius plots for the respiration data of some of the species studied show some such deviations from linearity over the experimental temperature range covered when regression lines are fitted (see Campbell, 1967). The two northern species show a distinct change of gradient (and thus of E_a) at the middle of the temperature range, while the three southern species show a reasonable straight line over most of the range with a possible break at low temperature for Crithmum.

Figs 4.6 and 4.7 show typical examples of the Arrhenius plots for Ligusticum scoticum, Mertensia maritima and Crithmum maritimum. The 5°C interval Arrhenius plots (6 points only) for each of these three species were of consistent form which was confirmed by the subsequent 'detailed', closer experimental temperature interval, experiments. Limonium binervosum has non-consistent Arrhenius plots and is discussed in detail later.

The points of each Arrhenius plot were initially treated as lying on a straight line and the relevant regression line was calculated and plotted graphically. Some lines showed no characteristics which could be said to deviate from straight, while others, for example Ligusticum, consistently had the furthest separated points on one side of the line and the middle points on the opposite side of the line, suggesting that

a curve or two straight lines would be a better fit. Where this apparent deviation from linearity applied the best fitting pair of regression lines, in terms of regression coefficient 'r', were found by splitting the line between successive points and using the lines with the best values of 'r'. While this method was not fully satisfactory for the 5°C interval (6 point) lines, it was much more so for the detailed temperature experiments which had 10 or more points to each graph.

Ligusticum has a break in gradient which occurs consistently between 15° and 20°C, with only one exception, for all the pretreatments used when pairs of regression lines were fitted as described above. When the 6 point lines are split in this way each of the two shorter regression lines has only one degree of freedom, so that while the values of E_a (as shown in Table 4.10) appear to be different for all the pretreatments, no reliability can be placed on the difference which depends on the break being real. However, when the four detailed experiments for Ligusticum were analysed in the same way they all showed a break in gradient, between 16° and 18°C for three, and between 14° and 16°C for the fourth. The Arrhenius plots for these detailed experiments are shown in Fig 4.8 and provide confirmatory and more substantial evidence that the break in gradient found is a real effect.

The individual values of E_a for both upper and lower temperature ranges were grouped relative to time and temperature of pretreatment separately (Table 4.14), but the resulting means show little variation when so grouped. The breaks for the 5°C interval experiments result in a lower E_a with a mean value for all treatments (Table 4.14) of 6.9 kcal.mol^{-1} above the break (at the higher temperature range) than below the break where the mean value is 13.6 kcal.mol^{-1}. The equivalent mean values for the four detailed experiments are 7.9 and 17.9 kcal.mol^{-1}.

A break of similar form is also found on the Arrhenius plots for

Mertensia (Fig 4.6) with the resulting lower E_a at the higher end of the experimental temperature range (Table 4.11). For this species the points for the 4 lowest temperatures gave better (i.e. greater) values of 'r' than those for the 3 lowest temperatures, suggesting that the break was between 20° and 25°C. This left only two points at the higher end of the temperature range for which it was not possible to calculate a regression line (no degrees of freedom) and the E_a values were calculated from the gradient of the line joining the two points. Though the break for Mertensia occurs less consistently than that for Ligusticum, only appearing for about half the pretreated plants, it must be remembered that plant material was limiting for this species. Where this was the case the volumes of gas measured on the Gilson respirometer were often so small as to be subject to greater error than usually acceptable, giving some erratic results (see below).

The single detailed experiment for Mertensia was also affected by limited plant material, but, despite this, the Arrhenius plot shows a break in gradient between 19° and 22°C as shown in Fig 4.9. This helps to confirm that the break in gradient is a real effect and is of similar form to that of Ligusticum, the other northern plant in the study.

When the E_a for the two parts of the temperature range are considered separately the values for the higher part of the range, from 25° to 30°C, are very erratic with values ranging from 3.6 to 20.4 kcal.mol^{-1} (Table 4.14). Some of this variation is created by single apparently erratic points on the graphs with the reservations discussed above, and the resulting mean E_a for all the pretreatments is 8.5 kcal.mol^{-1} (Table 4.14). The equivalent value for the lower temperature range is 11.0 kcal.mol^{-1}, and more reliability can be placed on this value. The E_a values for the single detailed experiment were 9.1 and 6.0 kcal.mol^{-1} for the lower and upper temperature ranges respectively.

While the scarcity of plant material for Mertensia may be one explanation for the less consistent appearance of breaks in gradient, it is apparent from Table 4.14 that the pretreatment temperature does affect the E_a for this species. There is a decrease in mean E_a for the overall range (probably the most reliable figure for comparison) from 13.9 kcal.mol^{-1} after 5°C pretreatments to 7.5 and 8.5 kcal.mol^{-1} after 20° and 25°C pretreatments respectively. This may also have an effect on the way in which any break in gradient is affected by pretreatment though no firm conclusions can be drawn from the available data.

Of the southern species, Crithmum maritimum is the only one for which the Arrhenius plots over the experimental range 5° to 30°C show any consistent indication of a possible deviation from linearity for all temperatures of pretreatment. On the 6 point graphs the value of $\log_{10}Q0_2$ for 5°C does not appear to lie on the straight line of the other 5 points for over 70% of the pretreatments: typical examples of this are shown in Fig 4.7. While the deviation of this single point is not significant in isolation, the consistency with which it occurs lends weight to the possibility that it may reflect a real temperature dependent effect. The individual overall E_a results and those for the experimental temperature range 10° to 30°C are given in Table 4.12 (it is not possible to calculate E_a below 10°C on the basis of one point) and from the corresponding means of Table 4.15, the mean E_a for the overall range is 10.3 kcal.mol^{-1} and that for the 10° to 30°C range is 11.2 kcal.mol^{-1}. The detailed experiments on Crithmum gave Arrhenius plots which appeared to confirm that there was a break in gradient which occurred at around 7°C, but since there are only three points on the lower temperature range line (shown in Fig 4.10) the existence of a break only becomes slightly more definite on this evidence. The data in Table 4.12 show the drastic reduction in E_a

below 7°C from 13.5 to 1.5 kcal.mol^{-1} for the 5°/49days pretreated plant: after 15°/48 days (detailed) the equivalent values were 11.0 to 0.3 kcal.mol^{-1}, with the break again around 7°C. For this species the pretreatment temperature has less influence on E_a than the length of the treatment: 1 week treatments have a mean E_a of 13.8 kcal.mol^{-1} and 2,3 and 4 week treatments have values lying between 10.2 and 10.5 kcal.mol^{-1} over the experimental temperature range of 10° to 30°C.

The Arrhenius plots for Limonium binervosum do not show, overall, any characteristics which could be said to deviate from a straight line. There is, however, an indication of two breaks in gradient on the graphs, which do not occur together in the present limited data. From E_a figures in Table 4.13, the 5°/ and 10°/2 weeks pretreatments on L.b.V and the 5°/ and 10°/3 weeks pretreatments on L.b.B show a break in gradient between 15° and 20°C or 20° and 25°C (Fig 4.11) similar to that shown by Ligusticum and Mertensia, all with slightly improved values of 'r' for the two shorter lines over that for the whole temperature range. This type of break is absent from any pretreatments at higher temperatures. After some higher temperature pretreatments, notably 20°/ and 25°/2 weeks and 25°/3 weeks for L.b.V and 20°/3 weeks for L.b.J (Table 4.13), there is a more definite break in gradient. This break (Fig 4.12) is between 10° and 20°C and is of a similar kind to that observed in Crithmum.

These breaks for Limonium each result in changes in E_a over the experimental temperature range of 5° to 30°C. The lower temperature (5° and 10°C) pretreated plants which show a 'northern type' break, with reduced E_a at the higher end of the temperature range, have widely varying individual values of E_a above and below the break, (Table 4.13) but the means of E_a (from Table 4.15) are 8.5 and 15.5 kcal.mol^{-1} for 5°C pretreated plants and 4.6 and 14.2 kcal.mol^{-1} for 10°C pretreated plants, with the higher temperature range figure quoted first in each case. The higher

temperature pretreated (20° and 25°C) plants have the'southern type' break with reduced E_a at the lower end of the experimental temperature range studied. The mean values of E_a here (from Table 4.15) are 16.0 and 9.1 kcal.mol^{-1} for 20°C pretreated plants, and 16.0 and 5.0 kcal.mol^{-1} for 25°C pretreated plants, again with values for the higher end of the temperature range quoted first. While all these means are based on very few measurements, the fact that the pattern of breaks is consistent for 2 and 3 week pretreated plants suggests that this is a real effect dependent on the temperature of pretreatment. The time of pretreatment alone (Table 4.15) has no influence on the resulting E_a.

The two detailed temperature experiments for Limonium both yielded straight line Arrhenius plots (Fig 4.13). One plant had been pretreated at 15°C for 17 days and the other at 5°C for one day only, with corresponding E_a values (Table 4.13) of 11.9 and 12.7 kcal.mol^{-1} respectively. On the basis of the other experiments, the 5°C pretreatment might have been expected to show a break, but one day is hardly long enough for the plant to have responded. Two out of the three other 15°C pretreated plants (Table 4.13) also gave straight line Arrhenius plots and the break was after 3 weeks at this temperature in the third. The 15°C pretreated plants appear to respond to experimental temperature in a way which is intermediate between that of plants exhibiting the 'northern type' or 'southern type' breaks and this probably results in the observed straight line. There are no apparent differences between the plants from the three different sources used.

Of the fifth species, the southern Glaucium flavum, only one plant survived and this was subjected to a detailed temperature experiment to gain maximum information. The resulting Arrhenius plot Fig 4.14, yielded a good straight line ($r = 0.997$) over the whole range from 4° to 31°C, with an activation energy of 12.7 kcal.mol^{-1} (Table 4.13).

Table 4.10 : Ligusticum scoticum. Activation energies (E_a) in kcal.mol^{-1} from Arrhenius plots.

Pretreatment	Lower temp. range E_a	(r)	°C	Higher temp. range E_a	(r)	°C	Whole temp. range E_a	(r)	°C
10°/ 6 days '76	14.51	(0.999)	5-15	4.71	(-)	20-25	11.05	(0.982)	5-25
15°/ 6 days "	13.73	(0.999)	"	10.20	(-)	"	12.61	(0.998)	"
20°/ 7 days "	15.58	(0.997)	"	7.99	(-)	"	13.36	(0.992)	"
25°/ 7 days "	12.85	(0.999)	"	8.15	(-)	"	11.03	(0.995)	"
10°/15 days "	15.20	(0.995)	5-15	10.03	(-)	20-25	14.38	(0.996)	5-25
15°/15 days "	12.78	(0.999)	"	3.30	(-)	"	8.90	(0.969)	"
20°/16 days "	11.83	(0.985)	"	10.80	(-)	"	12.30	(0.996)	"
25°/16 days "	14.07	(0.999)	"	2.67	(-)	"	9.17	(0.956)	"
5°/14 days '77	15.69	(0.996)	5-15	5.68	(0.999)	20-30	10.93	(0.976)	5-30
10°/15 days "	13.21	(0.996)	"	4.94	(0.990)	"	9.70	(0.979)	"
15°/15 days "	14.25	(0.984)	"	7.91	(0.979)	"	11.98	(0.989)	"
20°/16 days "	16.78	(0.999)	"	5.65	(0.991)	"	13.22	(0.978)	"
25°/16 days "	12.01	(0.999)	"	7.18	(0.996)	"	10.31	(0.993)	"
5°/20 days '77	13.21	(0.999)	5-15	10.73	(0.999)	20-30	12.80	(0.998)	5-30
10°/21 days "	12.51	(0.999)	"	8.80	(0.999)	"	11.19	(0.997)	"
15°/21 days "	13.69	(0.983)*	"	5.31	(0.999)	"	11.47	(0.980)	"
20°/22 days "	12.54	(0.996)	"	5.12	(0.984)	"	10.72	(0.983)	"
25°/22 days "	11.34	(0.997)	"	5.90	(0.985)	"	10.80	(0.987)	"
Detailed: 2°C experimental temp. interval									
5°/19 days	15.80	(0.999)	10-16	7.80	(0.992)	18-30	10.58	(0.984)	10-30
10°/28 days	22.86	(0.999)	10-14	8.14	(0.995)	16-30	10.57	(0.974)	"
15°/19 days	16.21	(0.998)	10-16	7.36	(0.990)	18-30	10.60	(0.979)	"
20°/28 days	16.46	(0.999)	10-16	8.36	(0.996)	18-30	10.45	(0.986)	"

*5-20° range gives a better straight line, E_a = 14.43 (0.993)

Values of E_a and 'r' calculated from data of Tables 4.2 and 4.3.

Notes: 1 - E_a calculated from gradient of regression line for the points of the Arrhenius plots in the temperature range indicated. The second place of decimals is not significant.

2 - 'r' is correlation coefficient for the relevant regression line (see Campbell, 1967). For two point lines, no value of 'r' can be calculated: shown by (-).

Table 4.11 : Mertensia maritima. Activation energies (E_a) in kcal.mol^{-1} from Arrhenius plots.

Pretreatment	Lower temp. range E_a	(r)	°C	Higher temp.range E_a	(r)	°C	Whole temp.range E_a	(r)	°C
5°/15 days '77	15.42	(0.995)	5-20	6.43	(-)	25-30	13.86	(0.992)	5-30
10°/16 days "	10.71	(0.969)	"	6.14	(-)	"	11.82	(0.986)	"
15°/16 days "	10.08	(0.973)	"	8.67	(-)	"	11.70	(0.988)	"
20°/17 days "	9.54	(0.999)	"	9.04	(-)	"	9.08	(0.999)	"
25°/17 days "	9.87	(0.997)	"	8.95	(-)	"	9.15	(0.997)	"
5°/27 days '77	15.27	(0.999)	5-20	6.93	(-)	25-30	13.05	(0.991)	5-30
10°/28 days "	10.61	(0.998)	"	20.44	(-)	"	12.61	(0.990)	"
15°/28 days "	8.85	(0.995)	"	15.95	(-)	"	10.26	(0.990)	"
20°/29 days "	7.52	(0.977)	"	5.81	(-)	"	6.99	(0.990)	"
25°/29 days "	7.77	(0.963)	"	9.01	(-)	"	7.91	(0.988)	"
5°/14 days '78	14.43	(0.995)	5-20	4.71	(-)	25-30	14.05	(0.981)	5-30
10°/14 days "	6.31	(0.781)	"	6.26	(-)	"	6.53	(0.919)	"
15°/14 days "	8.32	(0.987)	"	11.67	(-)	"	8.24	(0.994)	"
20°/14 days "	8.56	(0.984)	"	7.03	(-)	"	6.46	(0.969)	"
5°/21 days '78	17.79	(0.987)	5-20	5.73	(-)	25-30	14.60	(0.981)	5-30
10°/21 days "	15.73	(0.997)	"	3.61	(-)	"	13.72	(0.988)	"
Detailed : 3° experimental temp. interval									
20°/17 days '78	9.11	(0.958)	4-19	5.96	(0.997)	22-31	8.56	(0.986)	4-31

Values of E_a and 'r' calculated from data of Tables 4.4 and 4.8.

Notes as for Table 4.10

Table 4.12 : Crithmum maritimum. Activation energies (E_a) in kcal.mol^{-1} from Arrhenius plots.

	Lower temp. range			Higher temp. range			Whole temp. range		
Pretreatment	E_a	(r)	°C	E_a	(r)	°C	E_a	(r)	°C
5°/ 6 days	-			15.93	(0.996)	10-25	16.04	(0.999)	5-25
10°/ 6 days	-			13.59	(0.996)	"	11.34	(0.979)	"
15°/ 6 days	-			13.69	(0.997)	"	11.71	(0.985)	"
20°/ 7 days	-			12.11	(0.996)	"	11.19	(0.995)	"
25°/7 days	-			13.48	(0.996)	"	11.91	(0.990)	"
5°/15 days	-			11.49	(0.995)	10-30	11.07	(0.996)	5-30
10°/15 days	-			10.75	(0.997)	"	9.68	(0.994)	"
15°/15 days	-			10.10	(0.999)	"	9.53	(0.997)	"
20°/16 days	-			10.03	(0.996)	"	8.78	(0.985)	"
25°/16 days	-			10.32	(0.990)	"	8.94	(0.979)	"
5°/21 days	-			9.58	(0.988)	10-30	9.26	(0.992)	5-30
10°/21 days	-			11.00	(0.996)	"	10.96	(0.998)	"
15°/21 days	-			10.45	(0.996)	"	9.81	(0.994)	"
20°/22 days	-			9.38	(0.998)	"	9.22	(0.999)	"
25°/22 days	-			10.64	(0.997)	"	10.07	(0.996)	"
5°/28 days	-			10.30	(0.997)	10-30	9.70	(0.996)	5-30
10°/28 days	-			9.53	(0.988)	"	8.57	(0.984)	"
15°/28 days	-			10.39	(0.998)	"	9.75	(0.996)	"
20°/29 days	-			10.19	(0.993)*	"	9.16	(0.987)	"
25°/29 days	-			11.15	(0.995)*	"	9.75	(0.984)	"
Detailed: 2°C experimental temp. interval									
5°/49 days	1.56	(0.993)	3-7	13.52	(0.990)	7-29	12.33	(0.985)	3-29
15°/48 days	0.27	(0.946)**	3-7	10.97	(0.983)	7-29	10.79	(0.971)	3-29

*15-30° range gives a better straight line, 10-30° used for consistency.
**3° point not reliable, insufficient equilibrium time at start.
Values of E_a and 'r' calculated from data of Tables 4.5 and 4.6
(- no value can be calculated for single point below 10°C).

Notes as for Table 4.10

Table 4.13 : Limonium binervosum and Glaucium flavum. Activation energies (E_a) in kcal.mol^{-1} from Arrhenius plots.

	Lower temp. range			Higher temp.range			Whole temp.range		
Pretreatment	E_a	(r)	°C	E_a	(r)	°C	E_a	(r)	°C
Limonium binervosum									
5°/14 days B	21.47	(0.998)	5-15	10.85	(0.993)	20-30	12.04	(0.957)	5-30
10°/14 days B			(points erratic)				6.29	(0.896)	"
15°/14 days J			(straight line)				5.38	(0.974)	"
20°/14 days J			(points erratic)				6.57	(0.858)	"
5°/14 days V	14.53	(0.999)	5-20	10.13	(-)	25-30	13.56	(0.998)	5-30
10°/14 days V	16.67	(0.999)	5-15	8.22	(0.989)	20-30	13.17	(0.988)	"
15°/14 days V			(straight line)				13.13	(0.997)	"
20°/14 days V	7.56	(0.999)	5-15	15.17	(0.998)	20-30	11.95	(0.987)	"
25°/14 days V	1.69	(-)	5-10	12.87	(0.999)	15-30	9.15	(0.964)	"
5°/21 days B	10.66	(0.990)	5-20	4.66	(-)	25-30	9.32	(0.989)	5-30
10°/21 days B	11.76	(0.982)	5-20	1.09	(-)	25-30	9.77	(0.975)	"
15°/21 days J	10.38	(0.992)	5-15	16.59	(0.969)	20-30	10.27	(0.964)	"
20°/21 days J	10.56	(0.992)	5-15	16.88	(0.973)	20-30	10.02	(0.964)	"
25°/20 days V	8.41	(0.999)	5-15	19.09	(0.999)	20-30	11.76	(0.975)	"
Detailed 3° experimental temp. interval									
5°/ 1 day V			(straight line)				12.70	(0.995)	4-31
15°/17 days B			(straight line)				11.90	(0.993)	4-31
Glaucium flavum									
Detailed 3° experimental temp. interval									
5°/ 1 day			(straight line)				12.73	(0.997)	4-31

Values of E_a and 'r' calculated from data of Tables 4.7 and 4.8.

The letter B, J or V refers to the origin of the plant used as shown in Table 4.1 (page 77)

Notes as for Table 4.10

Table 4.14 : Mean values of activation energy (E_a) for the two northern species, Ligusticum scoticum and Mertensia maritima, considered relative to time and temperature of pretreatment separately.

Pretreatment	Activation energy (E_a), kcal.mol^{-1} Lower range	Higher range	Whole range
Ligusticum scoticum			
5° (all times)	14.45 ±1.24 (2)	8.20 ±2.52 (2)	11.86 ±0.93 (2)
10° "	13.86 ±0.61 (4)	7.12 ±1.35 (4)	11.58 ±0.99 (4)
15° "	13.61 ±0.30 (4)	6.68 ±1.50 (4)	11.24 ±0.81 (4)
20° "	14.18 ±1.19 (4)	7.39 ±1.30 (4)	12.40 ±0.61 (4)
25° "	12.57 ±0.59 (4)	5.97 ±1.19 (4)	10.33 ±0.41 (4)
1 week (all temps)	14.17 ±0.58 (4)	7.76 ±1.13 (4)	12.01 ±0.58 (4)
2 weeks'76 "	13.47 ±0.74 (4)	6.70 ±2.15 (4)	11.19 ±1.31 (4)
2 weeks'77 "	14.39 ±0.85 (5)	6.27 ±0.55 (5)	11.23 ±0.62 (5)
3 weeks "	12.66 ±0.40 (5)	7.17 ±1.11 (5)	11.40 ±0.38 (5)
All treatments (not detailed)	13.65 ±0.35(18)	6.95 ±0.59(18)	11.44 ±0.35(18)
Detailed	17.83 ±1.68 (4)	7.91 ±0.22 (4)	10.55 ±0.03 (4)
Mertensia maritima			
5° (all times)	15.72 ±0.72 (4)	5.95 ±0.48 (4)	13.89 ±0.32 (4)
10° "	10.84 ±1.92 (4)	9.11 ±3.82 (4)	11.17 ±1.59 (4)
15° "	9.08 ±0.52 (3)	12.10 ±2.11 (3)	10.07 ±1.00 (3)
20° "	8.54 ±0.58 (3)	7.29 ±0.94 (3)	7.51 ±0.80 (3)
25° "	8.82 ±1.05 (2)	8.98 ±0.03 (2)	8.53 ±0.62 (2)
2 weeks '77 all temps	11.12 ±1.09 (5)	7.85 ±0.64 (5)	11.12 ±0.90 (5)
4 weeks '77 all temps	10.00 ±1.42 (5)	11.63 ±3.82 (5)	10.16 ±1.21 (5)
2 weeks '78 all temps	9.40 ±1.75 (4)	7.42 ±1.50 (4)	8.82 ±1.79 (4)
3 weeks '78 all temps	16.76 ±1.03 (2)	4.67 ±1.06 (2)	14.16 ±0.44 (2)
All treatments (not detailed)	11.05 ±0.88(16)	8.52 ±1.08(16)	10.63 ±0.72(16)
Detailed	9.11 - (1)	5.96 - (1)	8.56 - (1)

Note - Means are calculated from individual values of Tables 4.10 and 4.11 and are expressed ±S.E. (standard error, see Campbell, 1967) with number of values used for each mean given in brackets.

Table 4.15 : Mean values of activation energy (E_a) for the two southern species, Crithmum maritimum and Limonium binervosum, considered relative to time and temperature of pretreatment separately.

Pretreatment	Activation energy (E_a), kcal.mol^{-1} Lower range	Higher range	Whole range
Crithmum maritimum			
5° (all times)	-	11.82 ±1.42 (4)	11.52 ±1.55 (4)
10° "	-	11.22 ±0.85 (4)	10.14 ±0.63 (4)
15° "	-	11.16 ±0.85 (4)	10.20 ±0.51 (4)
20° "	-	10.43 ±0.59 (4)	9.59 ±0.54 (4)
25° "	-	11.40 ±0.71 (4)	10.17 ±0.63 (4)
1 week (all temps)	-	13.76 ±0.61 (5)	12.44 ±0.91 (5)
2 weeks "	-	10.54 ±0.27 (5)	9.60 ±0.40 (5)
3 weeks "	-	10.21 ±0.31 (5)	9.86 ±0.32 (5)
4 weeks "	-	10.31 ±0.26 (5)	9.39 ±0.23 (5)
All treatments (not detailed)	-	11.20 ±0.38(20)	10.32 ±0.37(20)
Detailed	0.91 ±0.64 (2)	12.24 ±1.27 (2)	11.56 ±0.77 (2)
Limonium binervosum			
5° (all times)	15.55 ±3.16 (3)	8.55 ±1.95 (3)	11.64 ±1.24 (3)
10° "	14.21 ±2.45 (2)	4.65 ±3.56 (2)	9.74 ±1.99 (3)
15° "	10.38 - (1)	16.59 - (1)	9.59 ±2.26 (3)
20° "	9.06 ±1.50 (2)	16.02 ±0.85 (2)	9.51 ±1.57 (3)
25° "	5.05 ±3.36 (2)	15.98 ±3.11 (2)	10.45 ±1.30 (2)
2 weeks B & J	21.47 - (1)	10.85 - (1)	7.57 ±1.51 (4)
2 weeks V	10.11 ±3.41 (4)	11.60 ±1.52 (4)	12.19 ±0.81 (5)
3 weeks, B, J & V	10.35 ±0.54 (5)	11.66 ±3.66 (5)	10.23 ±0.41 (5)
All treatments (not detailed)	11.37 ±1.70(10)	11.55 ±1.81(10)	10.17 ±0.71(14)
Detailed	-	-	12.30 ±0.40 (2)

Note - Means are calculated from individual values of Tables 4.12 and 4.13 and are expressed ± S.E. (standard error, see Campbell, 1967) with number of values used for each mean given in brackets.

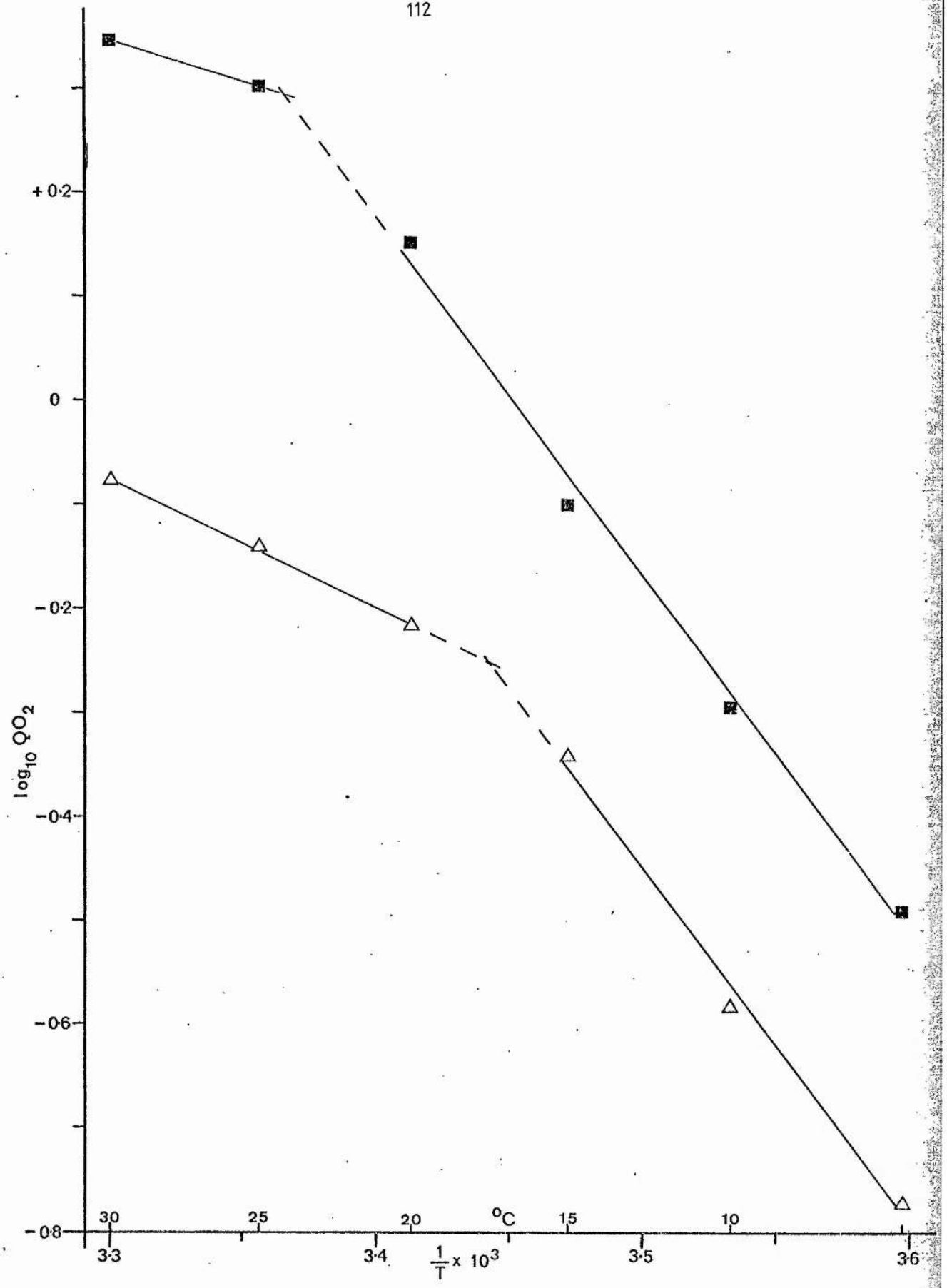


Fig 4.6: Typical Arrhenius plots for the two northern species; Ligusticum scoticum pretreated 5°/14 days △ , and Mertensia maritima pretreated 10°/21 days ■ . Plotted lines are regression lines.

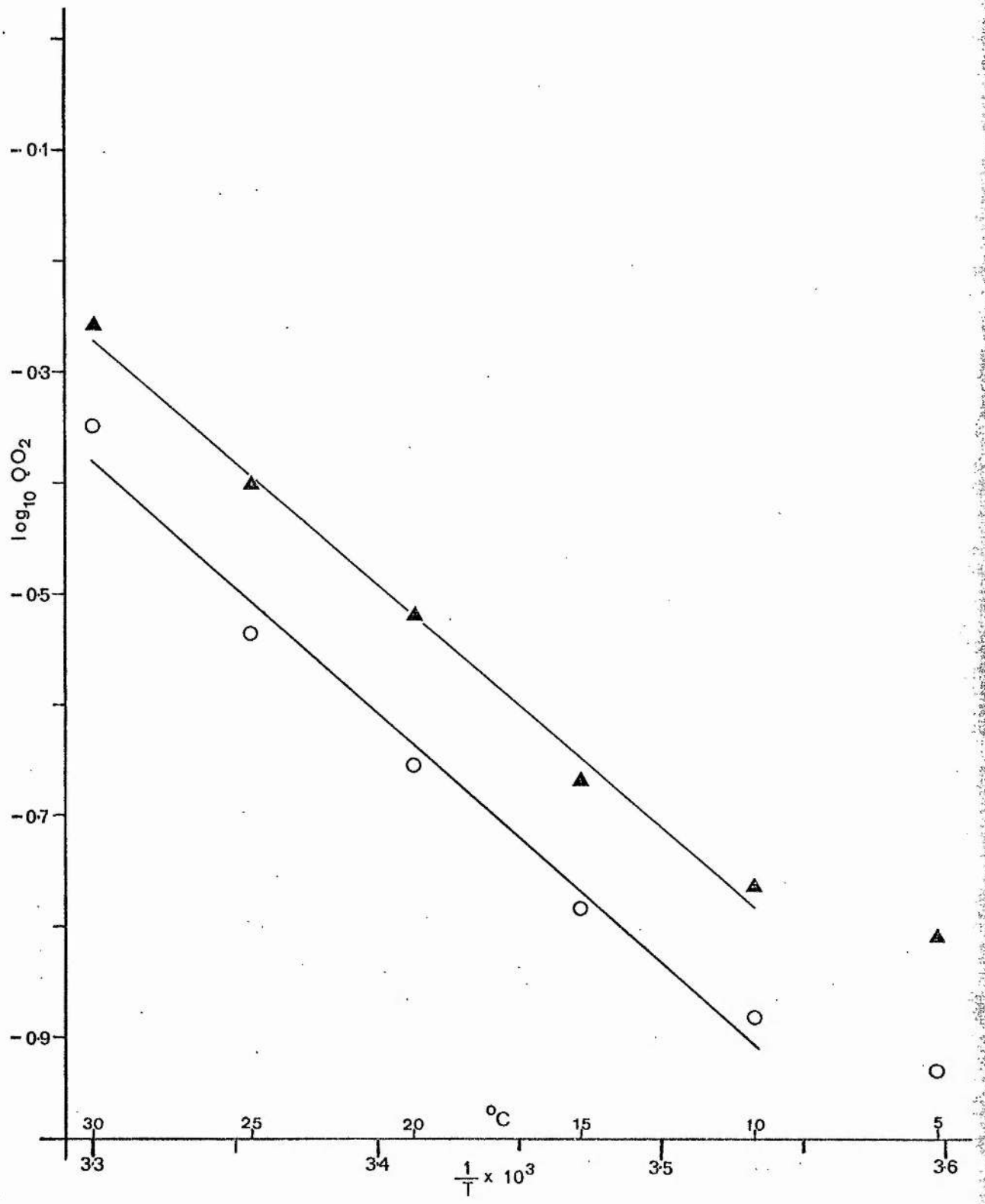


Fig 4.7: Two typical Arrhenius plots for the southern species Crithmum maritimum, pretreated 20°/16 days ▲ , and 25°/16 days ○ . Plotted lines are regression lines.

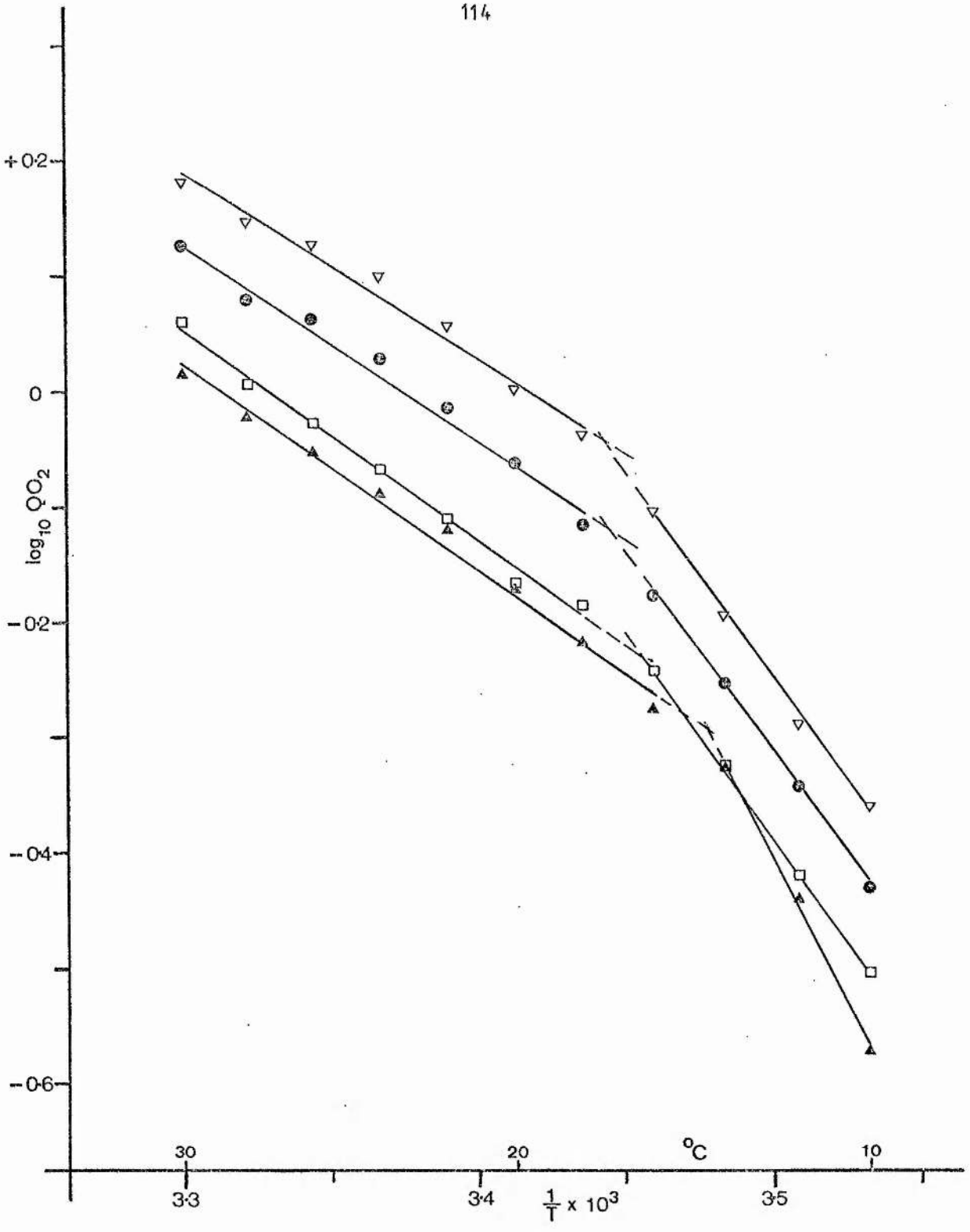


Fig 4.8: Ligusticum scoticum, Arrhenius plots of detailed ($2^{o}C$ interval) experiments. Pretreated at 5^{o}/19 days ● , 10^{o}/28 days ▲ , 15^{o}/19 days ▽ , and 20^{o}/28 days □ . Plotted lines are regression lines with 'breaks' between 14^{o} and $16^{o}C$ or 16^{o} and $18^{o}C$.

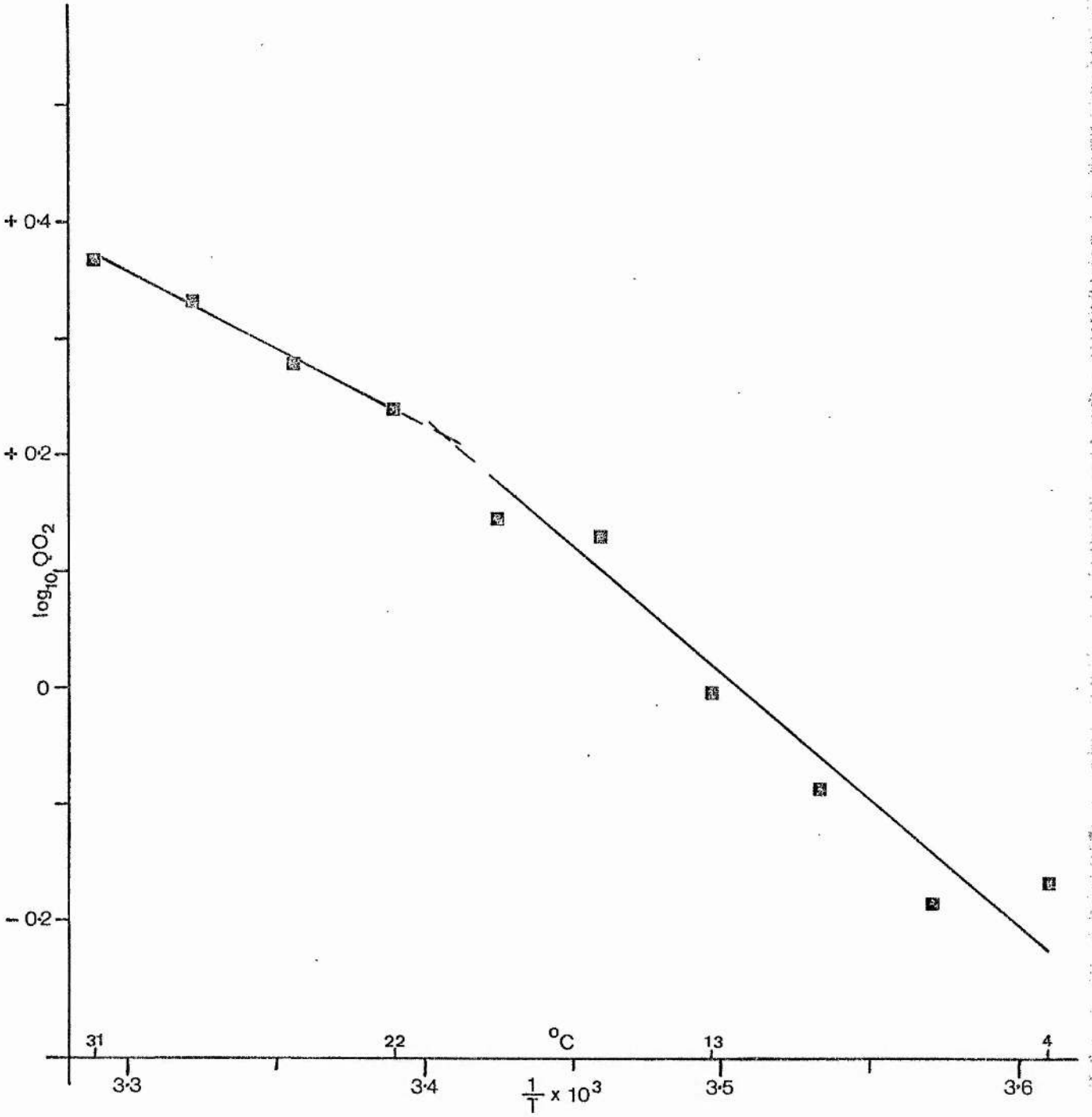


Fig 4.9: Mertensia maritima pretreated 20°/17 days. Arrhenius plot for detailed (3°C interval) experiment. Plotted lines are regression lines with 'break' in gradient between 19° and 22°C.

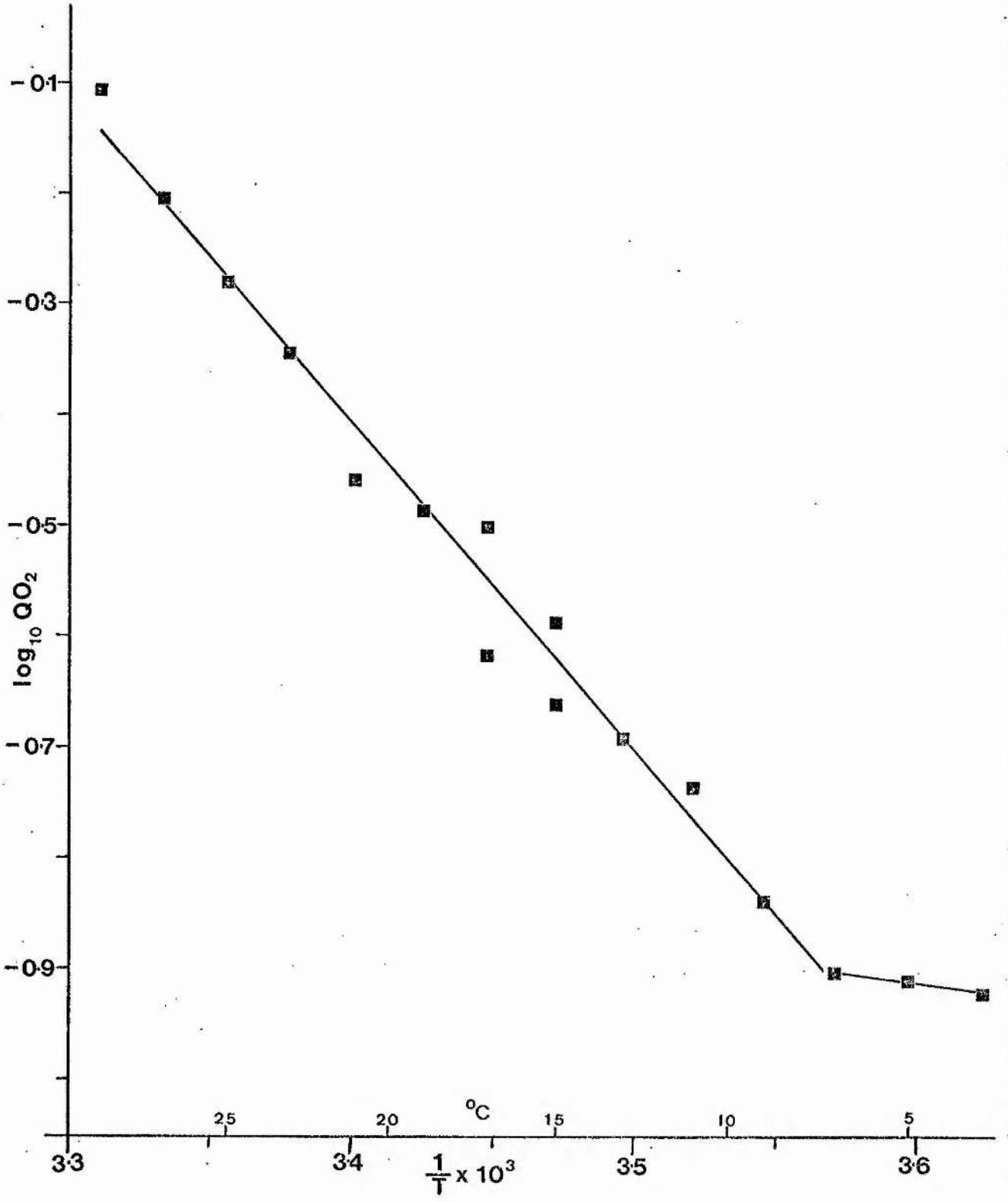


Fig 4.10: Crithmum maritimum pretreated 5°/49 days. Arrhenius plot for detailed (2°C intervals) experiment. Plotted lines are regression lines with 'break' in gradient at around 7°C.

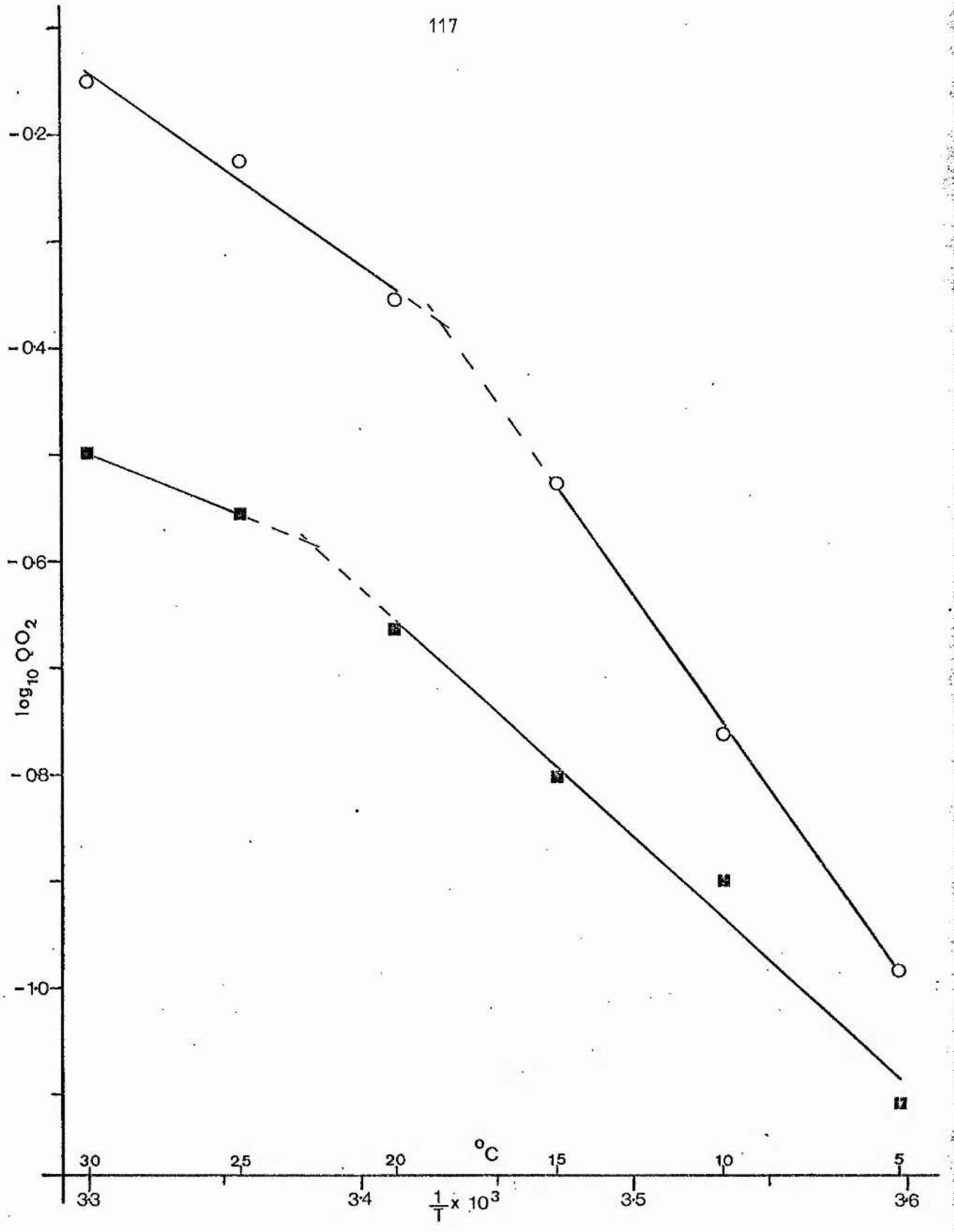


Fig 4.11: Limonium binervosum, Arrhenius plots for low temperature pretreated plants. L.b.V 10°/14 days ○ , and L.b.B 5°/21 days ■ , showing breaks at upper temperature range. Plotted lines are regression lines.

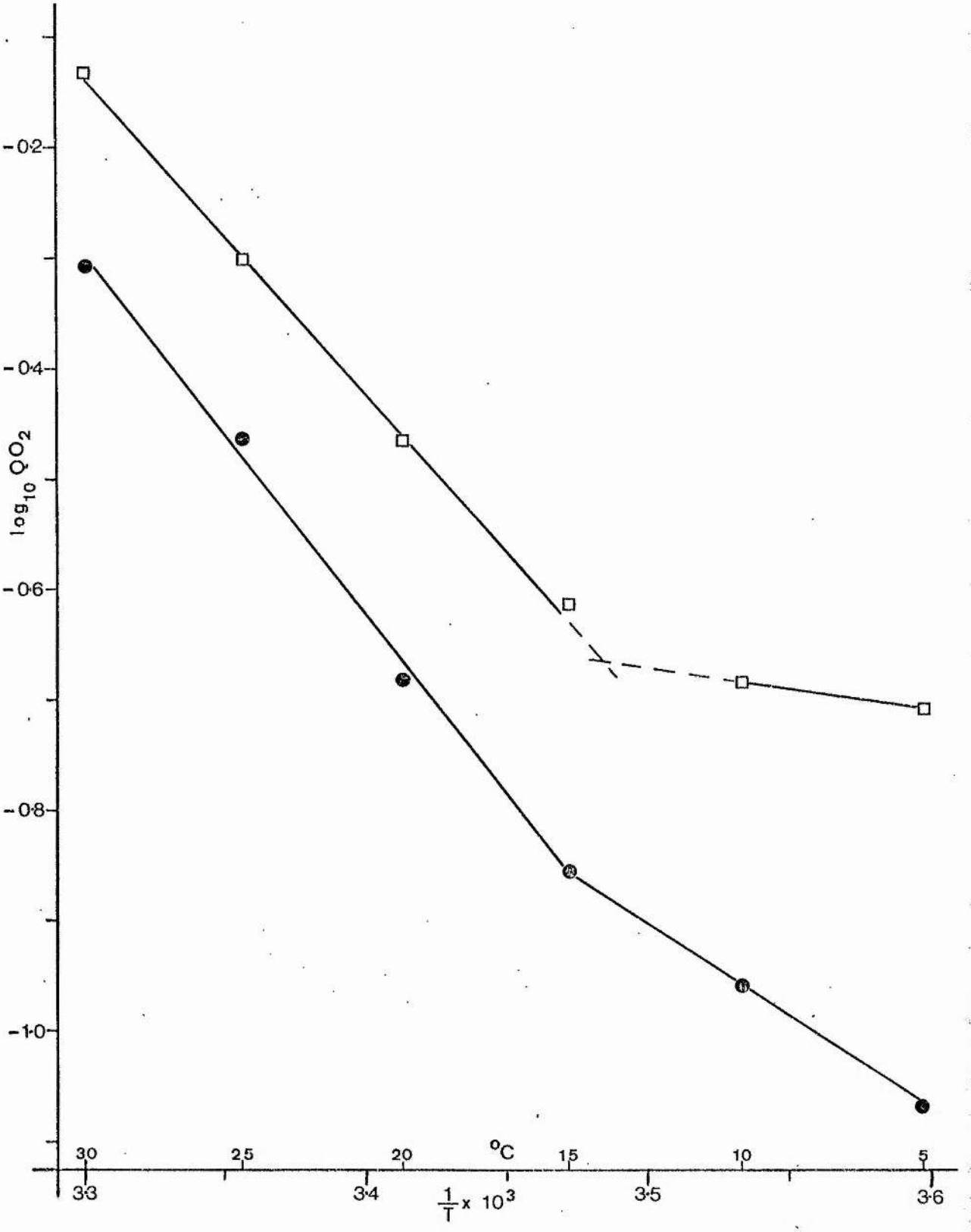


Fig 4.12: Limonium binervosum, Arrhenius plots for higher temperature pretreated plants. L.b.V 20°/14 days ● , and L.b.V 25°/14 days □ , showing breaks at lower temperature range. Plotted lines are regression lines.

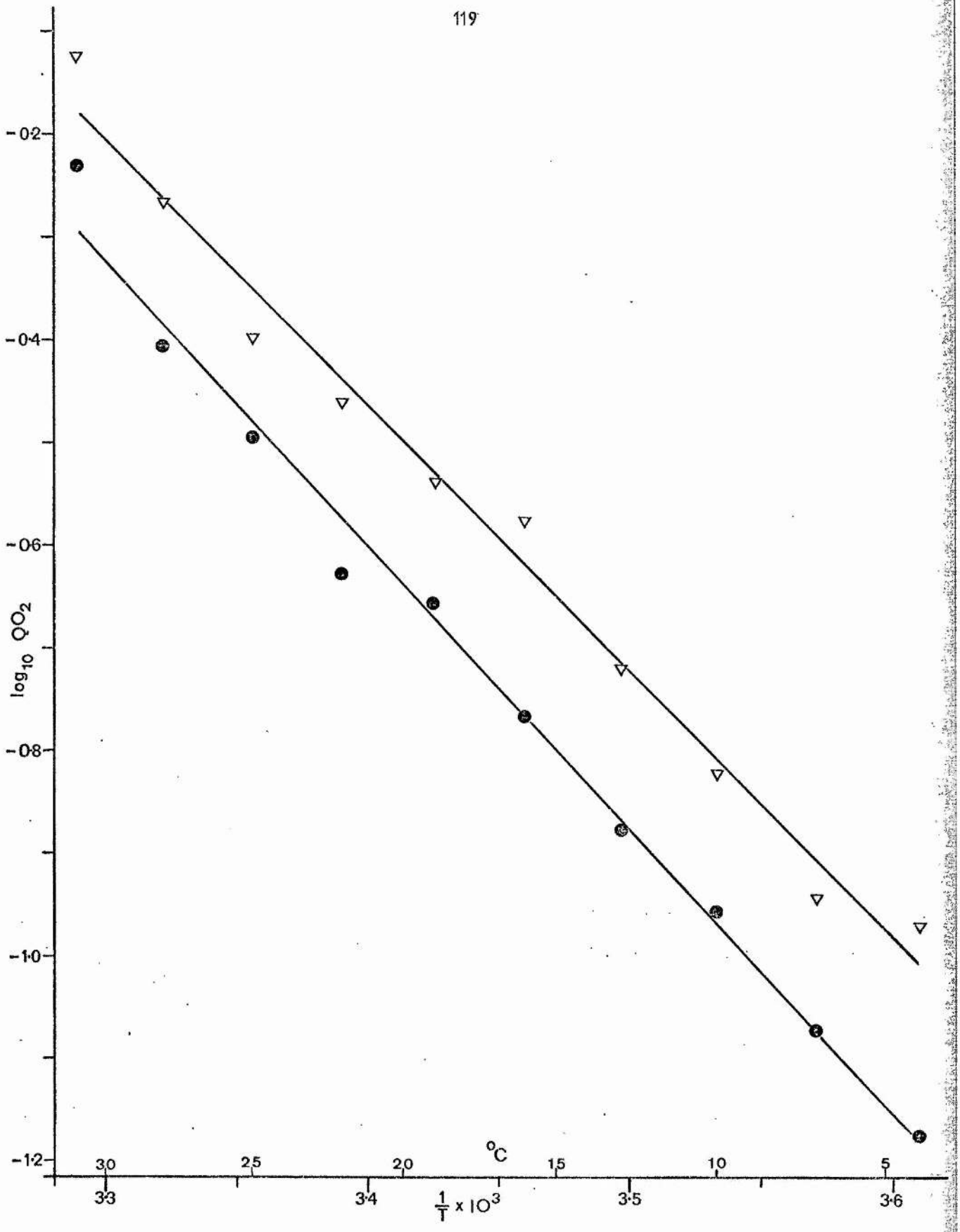


Fig 4.13: Limonium binervosum, Arrhenius plots of detailed (3°C interval) experiments. L.b.V 5°/1 day ● , and L.b.B 15°/17 days ▽ . Plotted lines are regression lines.

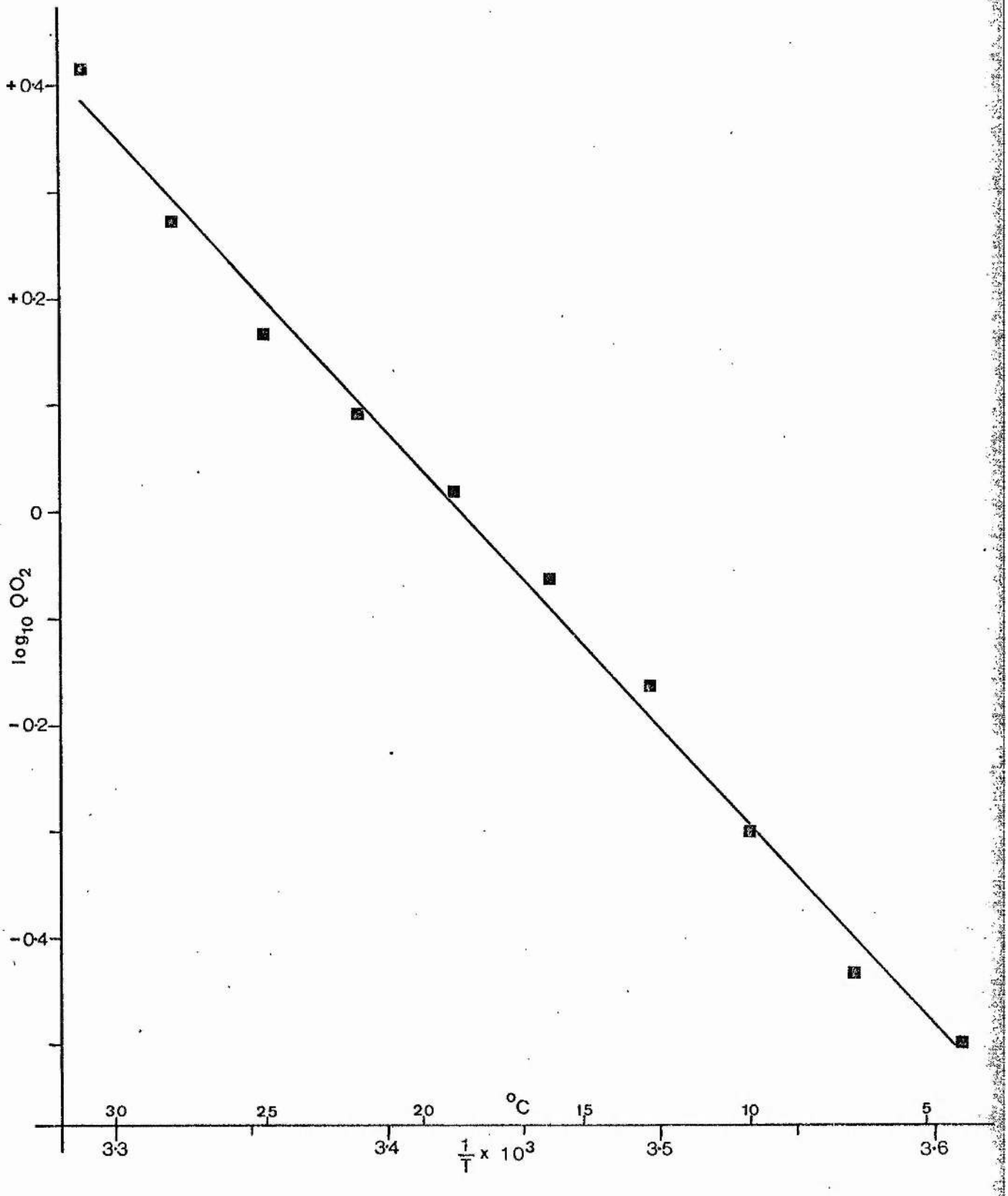


Fig 4.14: Glaucium flavum, Arrhenius plot of detailed (3°C interval) experiment. Plotted line is regression line.

DISCUSSION AND CONCLUSIONS

The root respiration rates of the northern and southern coastal species studied show different responses to changes in experimental temperatures, and also differences in the form of the corresponding Arrhenius plots and resulting energies of activation (E_a) for the various temperature ranges. These responses also vary to some extent with the temperature (and to a lesser extent time) of pretreatment to which each plant had previously been subjected, though this was different for each species studied.

The higher respiration rates (as QO_2) of the northern Ligusticum and Mertensia, and the rapid, almost linear, increase in respiration with increasing temperature up to 30°C, means that both plants appear to be well adapted to take maximum advantage of warmer spring weather by their rapid response. This theoretical advantage is confirmed by field observations, for Ligusticum at least, where after only a few days of noticeably warmer spring weather (in March or April in St. Andrews) a very great increase in foliage is rapidly apparent. This ability to respond so fast to improved temperature conditions is of even greater importance in the more northern parts of the range of these plants where the growing season may be reduced to a few weeks only , and a rapid response is imperative for the plants' survival. Conversely the high respiration rate may be the reason for these plants being unable to survive in southern Britain or beyond where the high respiration rates corresponding to the higher (southern) temperatures could result in excessive depletion. In order to form a more positive opinion on the effect of respiration on survival in the south for the northern plants, it would be necessary to have a detailed knowledge of the effect of temperature on photosynthesis and replenishment of carbohydrate in summer. This is beyond the scope of the present study and would be a logical extension of it.

The southern Crithmum and Limonium both have much lower rates of respiration which are only about one half of those of the two northern plants at any given (experimental) temperature in the range studied.

These two species have a similar rate of increase of respiration with temperature to the northern species, though the form of the response is closer to an exponential type curve than for the northern species. The respiration rate for these southern species shows only small increases at temperatures up to about 10°C, and thus the plants do not have the ability to respond to increasing temperature in the same rapid way as the northern plants at these lower temperatures. In fact a high rate (CO_2) and rapid response would probably be a disadvantage to these plants which encounter higher temperatures in their natural habitats than do the northern plants.

The limited study of Glaucium flavum yields respiration rates which are sufficiently high to be more akin to those of the northern species studied than to the southern group to which it belongs on the basis of its distribution. However, no further conclusions can be drawn on the basis of the single set of measurements. Further study is necessary, and especially on any effect which prolonged naturalisation in the north (St. Andrews botanic garden for 5 years) may have had on the physiology of respiration, either as expression of physiological plasticity or by selection.

For the southern species even more than the northern, a knowledge of the photosynthetic response to temperature would be the next priority for study, for comparison with the rates of respiration. All three southern species used in the present investigation retain at least some photosynthetic tissue throughout the winter; as leaves only for Glaucium and Limonium, and as leaves and green parts of the stem for Crithmum. This potential for replacement of at least small quantities of carbohydrate in winter must be of some importance to the plants for their winter survival, as Stewart and Bannister (1974) pointed out for Vaccinium uliginosum.

Respiration response to experimental temperature does vary with the pretreatment temperature (and to a lesser extent with time) for all the four main species studied. This is most marked for Mertensia, with a reduction in response to experimental temperature with increasing temp-

erature of pretreatment. There is a similar but less definite reduction in response in Limonium, but no clear pattern for Crithmum or Ligusticum when considering QO_2 values alone.

When the Arrhenius plot data are considered, the contrast between the northern and southern plants is even more noticeable, with the mid-range break in gradient of both Ligusticum and Mertensia, the low-range break for Crithmum, two breaks for Limonium, and straight line for the single Glaucium experiment.

The position of the break for Ligusticum, between 15° and 20°C, shows interestingly good correlation with the limiting July mean temperature isotherm for 59°F (15.0°C) shown by the distribution maps and discussed in Chapter 2 (page 12). Here it must be remembered that all experimental measurements were carried out on roots, and thus the air temperature of the maps will be of less direct relevance to the limitation of distribution than the soil and soil surface temperatures actually encountered. The experimental break for Mertensia between 20° and 25°C is further removed from its July air temperature limitation of 60°F (15.5°C) but the same reservations on the direct relevance of this to the temperature experienced by the roots and underground stems are also of importance here. Scott (1963a) points out that in direct sunlight the shingle around Mertensia (see photo 2.2, page 15) may get so hot as to cause the plants to wilt, and though they do recover later in the day(when cooler) the high temperatures reached by the shoot will certainly affect the underground parts, if only by transfer of heat with the normal movement of water through the plant. This transfer of heat from above ground parts to the roots will only be of importance to the northern plants in the summer, especially if the differences between leaf and air temperature are as great as the 22°C recorded by Salisbury and Spomer (1964) for alpine plants in sunlight. At times of the year when there are no above ground parts, then the root temperature will more closely reflect the soil temperature, and this will be the critical factor determining the plants' survival and distribution, as found by Higgins and Spomer (1976) and Anderson and McNaughton (1973) for various species.

The apparent critical temperature for the two northern species, expressed for each as the point where the break in the Arrhenius plot occurs and there is a change in E_a, may be very close to an actual critical temperature for root respiration. The plants do not die if subjected to higher temperatures than this for short periods of time, but in effect compensate for the otherwise rapid increase of $Q0_2$ to very high levels by the reduction in gradient of the Arrhenius plot which reflects a reduction in the actual rate of respiration and also in the loss of carbohydrate from what it would have been had there been no such break in gradient. A saving of up to 52% in the respiration carbohydrate loss at 30°C has been calculated for Ligusticum at the modified rate as compared with the extended lower range rate for the 10°/15 days '77 pretreated plants. This kind of saving is of maximum importance at these marginally suitable temperatures when the actual rates of respiration are so high, since it is these very high rates which will eventually bring about the depletion of reserves and subsequent death of the plant. The higher the temperature at which Ligusticum was pretreated, the smaller the percentage saving at 30°C appears to be, though this may be caused by overall progressive weakening of the plant. If death does not occur immediately, then it may occur by the next growing season due to insufficient reserves for the whole winter when no replenishment is possible, and by being at a general physiological disadvantage, especially if in competition with other species.

The reduction in E_a, reflected by slower rate of increase in respiration rates, at temperatures above the break in the northern species may reflect a compensation by the plants to reduce energy losses at these potentially unsuitable temperatures, or it may reflect a change in enzyme characteristics at these higher temperatures. Breaks in gradient of Arrhenius plots of similar form to those found for Ligusticum and Mertensia have previously been found for isolated enzyme systems of

various plant species which makes this the more likely explanation. In work of Lyons and Raison (1970) reviewed by Larcher et al (1973), Arrhenius plots of succinate oxidation by mitochondria showed breaks in gradient for chilling sensitive species but not for chilling resistant ones. In this case higher E_a is found at the lower temperature range, which the chilling sensitive plants will not tolerate for any length of time, the reverse of the case with Ligusticum and Mertensia. However, a temperature dependent change in enzyme activity directly related to the chilling sensitivity was demonstrated, which lends weight to the direct relationship between the break found in the present study and its relationship with temperature limitations on distribution, though here the measurements were for the whole system of respiration (as oxygen uptake) and not for a single enzyme. The parallel can be further drawn between the two systems in that measurable rates exist in both cases over the temperature ranges which are unsuitable for the plants concerned over long exposure times, definitely resulting in death in the chilling sensitive plants and almost certainly so for the two northern plants of this study. Since overall respiration was measured there is the possibility of involvement of two or more enzymes in the formation of the observed break, as would occur if two enzymes with intersecting Arrhenius plots were rate limiting, one above the break and the other below. The measured overall respiration rate depends on the rate limiting reaction and thus could show a break in Arrhenius plot if such a change-over in the rate limiting enzyme reaction occurred.

Of the three southern species studied, Crithmum and Glaucium show no upper temperature range change in the gradient of Arrhenius plots while Limonium shows a break at experimental temperatures between 20° and 25°C but only after 5°C and 10°C temperature pretreatments. However, in addition to its northern limit in Britain, Limonium binervosum also has a southern limit in west Spain or Portugal (at an unknown temperature)

and might thus be more likely to show this apparent limitation by higher temperatures (as do Ligusticum and Mertensia) than Crithmum or Glaucium which are both found throughout the much warmer Mediterranean region and have to tolerate higher temperatures than Limonium.

At the lower end of the temperature range, Crithmum shows a change in Arrhenius plot gradient at about 7°C, while after high pretreatment temperatures of 20° and 25°C, Limonium shows a similar break at between 15° and 20°C, both species having a lower E_a below the break than above it. Though occurring at different parts of the temperature range, these breaks are of the same form as each other but of a type seldom found previously. In the case of the enzyme fumarate hydratase in alkaline solution the Arrhenius plots for both the forwards and backwards reactions show a break of this type (Massey, 1953, cited in Dixon and Webb, 1964) though the break occurs at a range of temperatures from 22° to 32°C and no satisfactory explanation is offered. For the overall respiration (QO_2) of Crithmum and Limonium it seems most likely that this type of break results from changes in a single enzyme over the temperature range rather than an interaction between two or more enzymes as is possible for the northern species (discussed above). If only one enzyme is involved, then the temperature induced change in E_a may reflect a change in conformation even to the extent of partial denaturation of the enzyme protein at temperatures below the break, and if the latter is true then the plant may eventually die as lower temperatures are reached and a greater proportion of the relevant enzyme is rendered inactive. However, the break may represent different changes for the two southern plants, especially in view of the dependence on pretreatment temperature in Limonium for the appearance of the break.

Though Crithmum is limited by the 40°F (4.4°C) January mean isotherm or the 35°F (1.7°C) mean February minimum isotherm (see Chapter 2 page 26) the mature plants are apparently not killed by frost which does kill its

seedlings (Malloch, 1970) though the degree of frost is not mentioned. Experiments by Okusanya (1976) however, did show that plants of Crithmum showed good survival rates after being subjected to a temperature of -5°C for 6 hours while keeping the roots unfrozen, and 50% survival after treatment at -10°C for 2 hours. These experiments were carried out on young plants, apparently not in their winter condition, and thus possibly reflect survival potential against late and early frosts which are not generally too severe. In winter, even if not fully dormant, the plant is in a state of reduced metabolic activity having lost most of its leaves etc. and it may be that the break in Arrhenius plot found in the present study is correlated with the change to and from this less active state. Thus the respiration physiology and the way in which it is affected by temperature do not seem to play such an important part in the regulation of distribution of Crithmum as other factors like seedling survival and ability to set seed in 'bad' summers, as discussed by Okusanya (1976).

The connection, if any, between the temperature responses of Limonium and its distribution is not immediately clear, though the two breaks in gradient found after different pretreatments may reflect the possible dual temperature limitation on the species at its northern limit at least (discussed in Chapter 2, page 34). The restricted appearance of breaks on the Arrhenius plots which are pretreatment dependent, leads to the suggestion that Limonium may only change the characteristics of its respiration response to temperature slowly with alteration in temperature conditions. If the temperature increases very rapidly after the plant has been accustomed to the cold, then the upper temperature range break (and limitation) will come into play in a way which is not found when the plant has been conditioned to these higher temperatures. The converse may be true when the plant temperature is rapidly lowered after it has been acclimated to higher temperatures. The physiological mechanism of the respiration responses to temperature is not clear, though the

possession of a range of temperature sensitive respiratory isoenzymes could produce this kind of effect. The suggested slow response to changing temperature may be closely connected with the European distribution of Limonium binervosum, where it is an exclusively 'Atlantic' species, and thus usually only experiences relatively gradual changes in temperature conditions throughout the year which enable it to modify its responses at such a rate that it remains or becomes acclimatised to the prevailing temperature conditions at all times. While this explanation of the two breaks for Limonium appears to be in accordance with the distribution of the plant, it must be remembered that the number of treatments in which each break was found was small, and less confidence can thus be placed on the results for this plant than for the others discussed above.

The Arrhenius plot for the single plant of Glaucium is a straight line over the experimental temperature range studied.

Table 4.16 : Mean values of activation energies, with that for the temperature range appropriate to the distribution of each plant underlined.

Species	Activation energy, E_a, in kcal.mol^{-1}		
	Lower range	Higher range	Whole range
Ligusticum scoticum	13.65 ±0.35 (18)	6.95 ±0.59 (18)	11.44 ±0.35 (18)
Mertensia maritima	11.05 ±0.88 (16)	8.52 ±1.08 (16)	10.63 ±0.72 (16)
Crithmum maritimum	0.91 ±0.64 (2)*	11.20 ±0.38 (20)	10.32 ±0.37 (20)
Limonium binervosum	-	-	10.17 ±0.71 (14)
Glaucium flavum	-	-	12.73 (1)

* This value, from the detailed experiments, is not strictly comparable with others in the Table, but it has been included for comparison.

Values taken from Tables 4.14 and 4.15.

Over the individual temperature ranges closest to those experienced by each plant for most of the year in its natural habitat there is a

close similarity in the values of E_a for the overall respiration reaction (QO_2) for all the plants. These are summarised in Table 4.16 (above) and range from 13.6 kcal.mol^{-1} for Ligusticum to 10.2 kcal.mol^{-1} for Limonium. The whole temperature range value has been used for Limonium in view of the greater uncertainty (and complexity) of breaks, and for Glaucium since no break was found. The fact that these 'distribution associated' values of E_a are within such a restricted range is itself confirmation that the overall respiration process (measured as QO_2) is comparable for all the species studied, and also lends weight to the suggestion that the reduced E_a above and below breaks in Arrhenius plots (where appropriate) is indicative of some kind of 'non-typical' respiration response.

To summarise, the main fact to emerge from the work of this chapter is that there is good evidence for a direct correlation between distribution of the northern plants studied, Ligusticum and Mertensia, and their root respiration response to temperature. A similar dependence on temperature response is not unequivocally demonstrated for any of the southern plants, though there are possible indications of this for Crithmum and Limonium.

CHAPTER 5

EFFECT OF TEMPERATURE ON WHOLE PLANTS

INTRODUCTION

The information in this chapter on observed changes in morphology of whole plants of Ligusticum scoticum, Mertensia maritima and Limonium binervosum mainly resulted from incidental observations made on temperature pretreated plants subsequently used for the respiration experiments of Chapter 4. No observations were made on Crithmum maritimum or Glaucium flavum. Additional information on Ligusticum was gained from a separate experiment. Possible direct effects on the survival of the plants were then considered.

MATERIALS AND METHODS

1. Incidental observations on the effect of pretreatment at a range of temperatures.

The plants were those whose origins are given in Table 4.1 (page 77). As already described in Chapter 4, the plants were taken from the pots in which they had been growing, or freshly collected, and surplus sand was gently removed. They were then kept in air-filled polythene bags with some damp tissues in the temperature controlled cabinets in the dark. The pretreatments lasted for the lengths of time indicated in the Results section(as in Chapter 4). Observations on any changes in morphology of the plants were made, with varying detail, immediately before they were used for the respiration experiments.

2. Whole plant reaction of Ligusticum scoticum to pretreatment at a range of temperatures.

This was a separate attempt to quantify the whole plant response of Ligusticum when subjected to a range of constant temperatures, in addition to the information gained from the pretreated plants of Chapter 4.

The plants were kept in the dark, to make them comparable with earlier experiments, and so that no replenishment of carbohydrate by photosynthesis could occur. This restricted the effects observed to results of respiration and other related growth processes.

Previously collected plants in their winter state were washed under the tap, gently mopped dry and weighed. They were then planted in moist sand, two to a pot, in plastic pots, and one pot was placed in each temperature controlled cabinet, in the dark, at 5, 10, 15, 20 and 25°C. The experiment lasted for 8 weeks, starting in mid-January, and the pots were watered when necessary during this period. At the end of the experiment a record was made of the visual appearance of the plants and then they were again washed dried and weighed.

RESULTS

1. Incidental observations on the effect of pretreatment at a range of temperatures.

In considering these results it must be remembered that the conditions experienced during these experiments are far removed from those which the plants would experience in their natural habitats. This is particularly so for the southern species which would never be subject to long periods of continuous darkness.

Though the information in Table 5.1 is not complete, it does show that Ligusticum leaves become etiolated at any temperature above 5°C, at which temperature they remain green and still small enough to be protected by dead leaf bases. There are not great differences apparent in Table 5.1 between the treatments in terms of the amount of etiolation resulting from experiments up to 4 weeks, though the maximum elongation occurs after 15°C for 19 days. Further information is available for Ligusticum in Results 2 (below).

The information for Mertensia (Table 5.2) is more complete. 5°C pretreated plants remain in a condition similar to that observed initially while 10° and 15°C pretreated plants show progressively increasing etiolation of leaves of up to 5 cm in length after 4 weeks at 15°C. The 20° and 25°C pretreatments show progressively decreasing length of etiolated leaves relative to 15°C treated ones. In all cases only the terminal 3 or 4 buds showed any response to the temperature treatments. New lateral roots developed only occasionally, and then only on the larger plants.

Limonium plants retain green leaves throughout the winter and are thus more susceptible to the lack of light. The root morphology of the plants from the three origins (see Table 4.1, page 77) differed considerably at the start of the experiments, but this did not appear to affect leaf response to temperature. L.b.B. had long roots with hard fibrous laterals; L.b.J. had a woody primary root with newer laterals; and L.b.V had only a woody root/stem base. In all pretreatments at 5°C the plants were healthy looking and the leaves remained green as Table 5.3 shows. After 2 weeks, only those plants stored at 20° or 25°C

showed any yellowing and etiolation of the leaves, however, by 3 weeks there was progressively worse yellowing from 10° to 25°C pretreatment. The plants treated at 25°C for two and three weeks both had two new lateral shoots which were also etiolated.

2. Whole plant reaction of Ligusticum scoticum to pretreatment at a range of temperatures.

The measurements of weight as presented in Table 5.4 for the Ligusticum plants before and after storage were intended as a rough indication of the changes in weight due to respiration and growth in the dark. Alterations of internal water relations may have given misleading results in some cases, as for the percentage rise in fresh weight after 10° and 15°C pretreatments (1 plant in each case), when a reduction in weight might be expected. The highest percentage loss of weight was found in the three plants which died.

The observations on the morphology of the plants show a clear pattern (Table 5.4). With increasing temperature of storage from 5° to 15°C the length of etiolated leaves increased, and it decreased thereafter up to 25°C stored plants which did not survive the experiment after their initial elongation. 15°C stored plants had most new root growth and greatest elongation of leaves, and one plant even had two well developed inflorescences. Photo 5.1 shows the condition of all the plants at the end of the experiment.

All the plants which were still living at the end of the experiment were repotted and placed in an open cold frame outside in early April. All recovered well and some flowered and set seed.

Table 5.1 : Observations on changes in morphology under constant temperature conditions in the dark for Ligusticum scoticum.

Pretreatment °C		Leaf elongation
5°/14 days	('77)	Lvs. visible but not elongated.
10°/15 days	"	To 1 cm.
15°/15 days	"	To 1 cm.
20°/16 days	"	Elongated. (no measurement)
25°/16 days	"	
10°/21 days	"	Elongated. (no measurement)
15°/21 days	"	
15°/19 days	"	To 3 cm.
10°/28 days	"	Healthy, sprouting.
20°/28 days	"	Sprouting.

No changes in root morphology.

Table 5.2 : Observations in morphology under constant temperature conditions in the dark for Mertensia maritima.

Pretreatment °C		Leaf elongation	Roots
5°/13 days	('77)	Buds enlarged.	
5°/15 days	"	Buds enlarged.	
10°/16 days	"	To 1 cm, upper 3-4 buds only.	
15°/16 days	"	0.5-3 cm, upper 3 buds only.	
20°/17 days	"	To 1 cm. Top 3 buds.	
25°/17 days	"	To 0.5 cm.	
5°/27 days	('77)	Buds enlarged as for 5°15 days.	
10°/28 days	"	To 3.5 cm, top 3 buds. Lower buds no elongation.	3 new laterals, c.2 cm long.
15°/28 days	"	To 5 cm, top 4 buds. 5th bud to 1 cm.	
20°/28 days	"	To 0.5 cm, 2 buds.	
25°/28 days	"	To 1.5 cm, 3 buds.	4 small laterals.
5°/14 days)	('78)	Buds enlarged.	
10°/14 days)	"		
15°/14 days	"	To 3 cm.	
20°/14 days	"	To 3 cm.	
20°/16 days	"	To 5 cm.	
5°/21 days	"	Buds enlarged	
10°/21 days	"	To 0.5 cm.	

Table 5.3 : Observations on changes in morphology under constant temperature conditions in the dark for Limonium binervosum.

Pretreatment (°C)		Leaves
L.b.B	5°/14 days	Green and healthy looking.
L.b.B	10°/14 days	
L.b.J	15°/14 days	Green.
L.b.J	20°/14 days	Slight yellowing and elongation.
L.b.V	5°/14 days	Green, reddish centre.
L.b.V	10°/14 days	Green, small area of rot.
L.b.V	15°/14 days	Green, slightly limp.
L.b.V	20°/14 days	Yellowing, slight etiolation.
L.b.V	25°/14 days	2 new shoots, all etiolated.
L.b.B	5°/21 days	Green and healthy looking.
L.b.B	10°/21 days	Slight yellowing.
L.b.J	15°/21 days	Yellowing.
L.h.J	20°/21 days	Yellow, etiolated
L.b.V	25°/21 days	Etiolated and some rotting. 2 new shoots.

Table 5.4 : Ligusticum scoticum. Observations on the changes in morphology and fresh weight after constant temperature storage for 8 weeks in the dark.

	Initial Weight (g)	Weight at 8 weeks (g)	% change	Observations
5°	28.7	21.2	-26.1	Small leaves, no elongation.
5°	9.2	8.0	-13.1	
10°	22.0	14.7	-33.2	Dead, possibly damaged at start.
10°	33.9	37.5	+10.6	Leaves to 12 cm. Many new fibrous roots.
15°	48.8	58.9	+20.7	Leaves to 20 cm, 2 inflorescence initials. Many new fibrous roots.
15°	49.1	45.0	- 8.4	Leaves to 15 cm. Few new roots.
20°	32.1	22.4	-30.2	Leaves to 5 cm. Some original roots dead. Few new fibrous roots.
20°	14.9	11.4	-23.5	Leaves to 6 cm. Few new fibrous roots.
25°	15.3	9.0	-41.2	Dead. Leaves to 5 cm. before death.
25°	18.9	8.6	-55.5	

Photo 5.1 : Ligusticum scoticum. Response to temperatures of storage from 5° to 25°C in the dark for 8 weeks.

DISCUSSION AND CONCLUSIONS

In both northern species a maximum response interms of etiolation is observed after storage at 15°C in a way very similar to that shown by Ward (1976) for stored onions. Above this temperature the plants show less elongation but this is not fatal in any experiment except 8 weeks at 25°C for Ligusticum. The fact that the other 8 week dark treated plants recovered after the experiment suggests that fairly lengthy subjection to high temperatures is necessary to affect directly the survival of the plant. However, any prolonged high temperature will tend to weaken the plant by leaving it both desiccated and with less "in reserve" (carbohydrate etc.), and this may make it more susceptible to attack and death from other causes e.g. grazing damage or fungal infection. The fact that the two northern species have no leaves in the winter means that the effects observed in this experiment could be of direct relevance to the plant if it should be subjected to high temperatures early in the season before leaf growth and before significant lengthening of the hours of daylight in the higher latitudes of the plants' ranges.

Limonium, the only southern species for which observations are available, shows progressively greater etiolation lengthening and yellowing of leaves with increasing time and temperature of storage. No plant was kept for more than 3 weeks and no deaths occurred in this time. However, this plant has little scope for storage of reserves in its small rootstock, and by remaining green throughout the winter it probably relies on continuous photosynthetic replenishment for survival. Limonium does not show any decrease in response with increasing temperature as the two northern species do above 15°C, and this is in keeping with its southern status.

It is of interest to compare the responses of the plants with the results of Chapter 4. Both northern species, Ligusticum and Mertensia, show a break in gradient approximately between 15° and 20°C on the Arrhenius plots, and both also show reduced response of plants to temperature above 15°C. The southern Limonium has a break in Arrhenius plot gradient similar to that of Ligusticum and Mertensia but only after

storage at 20° or 25°C and the progressively greater etiolation of plants with increasing time and temperature reflects the generally straight line nature of Arrhenius plots. The growth of lateral shoots after 2 and 3 weeks at 25°C possibly represents an acclimation of this plant to higher temperatures related to the break in gradient mentioned above.

CHAPTER 6

EFFECT OF TEMPERATURE ON CARBOHYDRATE CONTENT OF ROOTS

INTRODUCTION

The carbohydrate status of a plant, in terms of starch and soluble sugar content, is of great importance for its survival since many of the vital physiological processes depend on the respiratory oxidation of sugars for the release of the energy necessary for these processes. The equilibrium between starch and soluble sugars is temperature dependent (see for example James, 1953) with higher temperatures resulting in displacement towards starch formation and lower temperatures in greater soluble sugar concentrations. While temperature is important in regulating this displacement, it also controls the rate of use of carbohydrate (as glucose) by respiration. In winter when some plants have no leaves, then respiration loss will be the main regulator of overall carbohydrate concentration, but when leaves are present there may be some photosynthetic replenishment of carbohydrate.

There have been many studies of carbohydrate status in plants with relation to temperature and also to the temperature related variables of time of year and altitude. For example, Mooney and Billings (1965) investigated the effect of altitude on the carbohydrate content of several North American alpine species and found that where the plants occurred naturally then there was more carbohydrate, due to greater concentrations of starch, at lower altitudes. They interpreted this as an ecological adaptation to the higher temperatures encountered. However, in transplant experiments of high altitude plants to lower altitudes, carbohydrate content was greater in plants remaining at higher altitudes than in those transplanted, owing to the intrinsically greater respiration rates of plants which originated from the cooler higher altitudes acting to their disadvantage in the warmer lower altitudes. The equivalent but opposite effect of starch content decreasing with decreasing temperature has been quantified by Glier and Caruso (1973) for Verbascum thapsus L.. They

suggest, in common with others, that the increased soluble sugars present after low temperature treatment help the plant to withstand cold or freezing damage, and may also help to promote rapid growth in the spring without the necessity of conversion from stored starch.

Stewart and Bannister (1973) monitored the total carbohydrate concentration in Vaccinium uliginosum throughout the year, and found that they were able to explain the cyclical changes observed in terms of the respiratory loss and photosynthetic gain of carbohydrate by direct relation with light and temperature conditions at any time of year. This particularly shows the importance of the relatively fine balance between loss and gain of carbohydrate for the potential survival of a plant, and most especially in winter when assimilation is at its lowest.

In addition, Green and Ratzlaff (1975) have related the amount of soluble sugar in cereals with their winter cold hardiness. The level of sugar increased with increasing time of hardening for all cultivars used, but the two less hardy cultivars of wheat tested showed higher levels of soluble sugars than the two hardy ones after the same period of cold hardening.

In view of the above considerations, determinations of carbohydrate, in terms of starch and soluble sugar content, were made on the pretreated root material remaining after removal of samples for the respiration experiments of Chapter 4, to see if this would provide additional information relevant to the survival and distribution of the plants. Enough root material was available only for Ligusticum, Mertensia and Crithmum for starch and soluble sugar determinations, and for Ligusticum only, for individual sugars determination. Samples of fresh (i.e. not pretreated) root material were available for Ligusticum alone and from results obtained for this species down to 10°C pretreatment, it appeared that for all species used, the 5° pretreatment for the shortest time was probably the figure which differed least from that of fresh material, especially since all the plants had been collected in winter.

METHODS

1. Starch estimation.

The iodate/iodide method used was that described by Allen (1974) Chemical Analysis of Ecological Materials, section 42.5.

Between 150 mg and 250 mg of freeze dried, ground plant root material was accurately weighed in a test tube. 5.0 ml of water and 200 mg of fine washed sand were added and the contents boiled for 15 minutes, in a water bath, to gel the starch. After cooling, 5.0 ml of 60% perchloric acid ($HClO_4$) was added rapidly while stirring. The plant tissue was ground against the side of the tube, using a glass rod, intermittently for a further 20 minutes and then the extract was transferred quantitatively to a 100 ml volumetric flask, diluted to volume with distilled water, mixed and allowed to settle. Aliquots of this extract (see below) were analysed.

Each aliquot was transferred to a 50 ml volumetric flask, and after addition of a few drops of phenol red indicator (0.1% in industrial spirit) was neutralised by dropwise addition of sodium hydroxide (1.0M NaOH) until the indicator turned to deep red. Acetic acid (10% CH_3COOH) was added dropwise until the colour was just destroyed, and then a further excess 2.5 ml was added. After addition of 0.5 ml of potassium iodide (10% KI), the colour was developed by adding 5.0 ml of potassium iodate (0.0125M KIO_3) with thorough shaking. The assay solution was then made up to volume (50 ml) with distilled water and the orange to brown colour was measured spectrophotometrically at 680nm (red filter) against a distilled water blank.

A standard solution of pure starch (1 ml equivalent to 1 mg) was made up in the same way as for the plant tissue extracts, and aliquots of this were used to construct a calibration curve for the experimental analyses. Care was taken to choose sample aliquots which yielded colours and thus readings on the straight line part of the graph; up to about 1.0 mg of starch per assay. Determinations were carried out on three different aliquot volumes and the results from these were averaged to improve the precision. Sample replication was not possible owing to the small quantities of plant material available.

2. Total soluble sugar estimation

The anthrone method described by Allen (1974) section 41.4 was used.

Though 50 mg of dry plant material was suggested as a suitable sample size, it was found that this was too much for the plants under investigation and 10-20 mg was more suitable, to avoid dilution of extracts.

The accurately weighed sample was boiled gently with 30 ml of distilled water for 2 hours in a covered conical flask and was topped up as necessary. After cooling, the extract was filtered through a Whatman No.44 paper into a 50 ml. volumetric flask, washed through, and then made up to volume when cool. Filtration was slow, possibly due to extraction of oligosaccharides which might be expected to impede filtration. Determinations were carried out immediately on these solutions since they will not keep overnight.

2.0 ml of extract was pipetted into a boiling tube and 10 ml of anthrone reagent (see below) was added rapidly with mixing, with the tube in an ice-cold water bath. The assay solution was boiled in a water bath in a dark fume cupboard for 10 minutes. It was then cooled and the resulting green or blue colours measured at 625 nm against a distilled water blank.

A standard solution of glucose (1 ml equivalent to 0.25 mg) was made up and diluted to yield a range of standards which were assayed in the same way as above for the construction of a calibration curve up to 0.3 mg of glucose per assay, over which range a straight line was obtained. The measure of total soluble sugar obtained is in terms of 'glucose equivalent sugar'.

Once again, assays were carried out on two aliquots, and the results were averaged to improve precision, but replication of samples was not possible owing to the very small amounts of dried root material available.

Anthrone reagent

Add 760 ml of concentrated sulphuric acid (H_2SO_4 98% Analar) to 330 ml of distilled water, keeping cool while mixing. When cold dissolve 1.0 g anthrone and 1.0 g thiourea in this using a magnetic stirrer. Keep in the dark in a refrigerator for 2 hours before use. The anthrone is the colour producing agent and the thiourea is added to stabilise the rather easily decomposed anthrone.

3. Characterisation and determination of sugars by Gas Liquid Chromatography.

The method used was based on that of Sweeley et al (1963) and Ellis (1969), in which the sugars are converted to volatile silated derivatives before gas chromatographic determination.

The sugars from an accurately weighed 0.1g portion of freeze dried root material were extracted 3 times with 2 ml volumes of boiling 80% ethanol and 3 times with 2 ml volumes of boiling 60% ethanol. The extracts were bulked by filtering into a thick walled glass tube, then taken to dryness in a vortex evaporator under vacuum at 45°C. If not analysed immediately, the dry extracts were stored in a desiccator over phosphorus pentoxide.

The dry extract was redissolved, quantitatively, in 1.0 ml of dimethyl sulphoxide (DMSO, $CH_3.SO.CH_3$) and 0.2 ml of this was measured with a micropipette into a "cherry bottle" (a ca. 1 ml flask with 0.02 ml graduations on the neck.) After addition of a further 0.2 ml of DMSO, to rinse down the extract, 0.2 ml of hexamethyldisilazan (HMDS, $C_6H_{19}NSi_2$) followed by 0.1 ml of trimethylchlorosilane (TMS, C_3H_9ClSi) were added, the bottle was stoppered to prevent entry of water from the air and the contents mixed thoroughly on a mechanical shaker for 5 minutes.

After standing overnight two phases appear, the lower being DMSO, and the upper HMDS containing the trimethylsilated sugar derivatives. DMSO was then added to the lower phase with a syringe to displace the upper phase into the graduated neck of the "cherry bottle" and this volume, usually about 0.25 ml, was noted. A 1 µl aliquot of this upper phase was taken with a microsyringe and injected into the Gas Chromatograph which was fitted with a 1% SE 52 diatomite CQ column. This was programmed to run 2 mins at 130°C, then to rise at $6°.min^{-1}$ to 260°C, this temperature being held for a further 15 mins to flush out any less volatile compounds.

Standard solutions of the sugars expected to be present, dissolved in DMSO, were treated in the same way and a range of different sized aliquots from the upper phase was used to construct a separate calibration

graph for each sugar, since the proportion of each sugar standard recovered as its trimethylsilyl derivative was different for each one.

Amounts of each sugar in the sample aliquots were obtained by calculation of peak area (height from baseline x width at $\frac{1}{2}$ height) and reference to the relevant calibration graphs drawn up in the same way.

RESULTS

1. Starch

The northern species, Ligusticum shows concentrations of starch in the roots (Table 6.1) from 0.3% to 5.8% of the dry weight with the maximum after pretreatments at 10°C and the minimum after 25°C, except after 1 week where the pretreatment time was possibly insufficient for full equilibrium to have been reached. The results for 2 weeks '76 are slightly higher than the others but the same trend is shown. Amounts of starch after 5°C pretreatments are lower than those for 10°C in all instances where both were measured.

The starch contents of the second northern species Mertensia (Table 6.2) were all less than 1% and at these values were insufficiently precise, owing to low sample weights used, for any pattern to be noted. There was, however, no further dry root material available to enable determinations to be carried out on larger sample weights.

The southern species, Crithmum, shows a maximum starch content, relative to dry weight, of up to about 15% (Table 6.3) at 10° to 20°C with the maximum in every time group at 15°C becoming more marked after longer storage times. In contrast to Ligusticum this trend is shown after 1 week, suggesting that Crithmum is able to respond faster to temperature of pretreatment.

Table 6.1 : Starch content of roots of Ligusticum scoticum after various pretreatments.

Pretreatment temperature °C	% starch (relative to dry weight) 1 week '76	2 weeks '76	2 weeks '77	3 weeks '77	Detailed (3 and 4 weeks)
5°	-	-	1.3	2.5	2.2
10°	3.2	5.8	3.3	3.3	2.6
15°	2.5	2.7	1.5	1.1	1.7
20°	2.5	2.2	0.6	0.7	0.7
25°	3.1	0.4	0.4	0.3	-

Table 6.2 : Starch content of roots of Mertensia maritima after various pretreatments.

Pretreatment temperature °C	% starch (relative to dry weight) 2 weeks '77	4 weeks '77
5°	0.5	0.2
10°	0.2	0.4
15°	0.7	< 0.1
20°	0.6	0.1
25°	< 0.1	0.2

Notes 1 - Root material was that remaining after samples taken for respiration experiments of Chapter 4.

2 - First place of decimals not always significant, but is included for later averaging.

Table 6.3 : Starch content of roots of Crithmum maritimum after various pretreatments.

Pretreatment temperature °C	% starch (relative to dry weight) 1 week	2 weeks	3 weeks	4 weeks
5°	3.7	9.9	6.4	3.3
10°	10.1	15.3	10.6	1.0
15°	11.2	15.6	13.2	14.5
20°	10.0	8.3	10.1	7.6
25°	3.6	7.8	5.3	6.1

Notes : as for Tables 6.1 and 6.2.

2. Total soluble sugars

The northern Ligusticum shows concentrations of between 33% and 46% soluble sugars (Table 6.4), expressed as glucose equivalent of the dry weight. While there is variation in the sugar content with different pretreatment conditions there are no distinct trends which can be related to either time or temperature of pretreatment. The differences observed may be a reflection of the natural variability between the sugar levels in the individual plants used.

Mertensia, the other northern species, has very high concentrations of soluble sugars in its roots (Table 6.5) up to 81% after pretreatment at 5°C for 2 weeks. There is a decrease in sugar content, to a minimum of 51%, with increasing pretreatment temperature, and this is most marked after pretreatment at 20° and 25°C for 4 weeks.

The southern Crithmum shows maximum sugar contents (Table 6.6) after pretreatment at 10° or 15°C with maximum levels of around 50% of the dry weight. There is a general decrease in sugar content with increasing temperature of pretreatment above the temperature of the maximum content. The plant material for Crithmum was all collected at the same time and place and can be considered partially clonal, and this should have helped to reduce differences in results due to natural variation making comparisons more valid.

The anthrone method is sometimes considered to give unrealistically high levels of soluble sugars due to exhaustive water extraction of the plant material and also due to possible hydrolysis of oligosaccharides with the strong acid of the anthrone reagent.

3. Starch and Sugars considered together

The amount of total soluble sugar in Ligusticum (Table 6.4) is very similar to that in Crithmum (Table 6.6) whereas the starch contents (Tables 6.1 and 6.3) are considerably different. It is of interest to express these concentrations as ratios of the means of each at the various pretreatment temperatures used (all times) and also to obtain an arbitrary

measure of 'total carbohydrate' at any temperature by summation of the means, both in order to see if additional information can be obtained by treating the results in this way. Means and derived values are given in Table 6.7 and include, for completeness, the corresponding values for Mertensia (original Tables 6.2 and 6.5). The values for the ratio emphasise the considerably higher sugar : starch ratios for the northern Ligusticum in relation to those for the southern Crithmum, and show that there is a minimum ratio at 10° for Ligusticum. Mertensia, the other northern species has an even greater sugar to starch ratio, due to higher levels of sugars and lower levels of starch than for Ligusticum.

The corresponding 'total carbohydrate' values (Table 6.7) show that for Ligusticum there is a small decrease in total carbohydrate with increasing temperature of pretreatment, while Crithmum has increasing total carbohydrate up to 15°C pretreatment and a subsequent decrease. This latter result, if real, can only be explained by possible breakdown of some other storage material not measured by either of the methods used, which increases either starch or soluble sugar, or both, and so increases the apparent total. Total carbohydrate for Mertensia is largely a measure of the soluble sugar content with the trends already discussed.

In considering the starch and sugar concentrations, both above and in the two previous sections, it should be mentioned that all measurements have been made relative to dry weight of root. After any length of time the weight loss of the root due to loss of carbohydrate by respiration, will make sugar content figures appear higher than they should. This will be of most importance after the longest times of pretreatment, 3 and 4 weeks, when greatest dry weight losses will have occurred.

It is also of interest here to compare the actual losses of carbohydrate measured in these experiments with the losses calculated from the respiration data of Chapter 4. The mean percentage loss of dry weight at 5° and 25°C was calculated (as glucose) from the respiration rates of Table 4.9 (page 95) and this information is presented in Table 6.8 along with the resulting times for loss of 50% of the dry weight, a figure which

would be bound to affect all three species considered, and especially in view of their measured carbohydrate contents (Table 6.7). The calculated times of 70 and 50 days for loss of 50% of dry weight in Ligusticum and Mertensia at 5°C are of the right order for survival of these plants through the winter, and when the possibility that measured respiration rates were up to twice as great as those in the field the 50% loss time becomes up to 140 and 100 days for the two plants. This is a very similar time to that which these species remain leafless in the British winter. Both lower and higher temperatures may be encountered in winter by the northern plants, increasing or reducing the length of time required to lose 50% of the dry weight and possibly affecting the plants' survival. The effect of carbohydrate loss on Crithmum will be less important for survival since this loss is slower than in the northern species and the plants retain leaves in winter which enables some replenishment of carbohydrate to offset any losses.

Table 6.4 : Total soluble sugar content (glucose equivalent) of roots of Ligusticum scoticum after various temperature pretreatments.

Pretreatment temperature °C	% glucose equivalent sugars (relative to dry weight)				
	1 week '76	2 weeks '76	2 weeks '77	3 weeks '77	Detailed (3 & 4 weeks)
5°	-	-	45.8	41.8	41.9
10°	44.1	34.4	42.7	33.7	47.2
15°	40.4	37.6	45.4	34.5	40.6
20°	44.8	33.4	39.5	41.6	40.9
25°	39.5	35.2	36.1	32.9	-

Table 6.5 : Total soluble sugar content (glucose equivalent) of roots of Mertensia maritima after various temperature pretreatments.

Pretreatment temperature °C	% glucose equivalent sugars (relative to dry weight)	
	2 weeks '77	4 weeks '77
5°	81.3	72.0
10°	69.5	76.6
15°	72.3	76.2
20°	70.8	66.6
25°	61.9	51.5

Notes 1 - Root material was that remaining after samples taken for respiration experiments of Chapter 4.

2 - First place of decimals not always significant, but is included for later averaging.

Table 6.6 : Total soluble sugar content (glucose equivalent) of roots of Crithmum maritimum after various temp. pretreatments

Pretreatment temperature °C	% glucose equivalent sugars (relative to dry weight)			
	1 week	2 weeks	3 weeks	4 weeks
5°	37.6	37.8	39.8	37.9
10°	52.2	42.7	53.7	34.7
15°	37.8	44.4	39.1	57.1
20°	31.0	36.2	34.7	30.2
25°	32.0	37.1	32.6	31.0

Notes: as for Table 6.4 and 6.5.

Table 6.7 : Ligusticum scoticum, Mertensia maritima and Crithmum maritimum: mean values for starch and total soluble sugar content related to temperature of pre-treatment. 'Total carbohydrate' and the sugar:starch ratio are derived directly from these values.

	Percentage of dry weight			
	Starch	Soluble sugars	Total carbo-hydrate	Sugar: Starch ratio
Ligusticum scoticum				
5°	2.0 ±0.3 (3)	43.2 ±1.3 (3)	45.2	21.5
10°	3.6 ±0.5 (5)	40.4 ±2.7 (5)	44.0	11.1
15°	1.9 ±0.3 (5)	39.7 ±1.8 (5)	41.6	21.1
20°	1.3 ±0.4 (5)	40.0 ±1.9 (5)	41.3	29.9
25°	1.0 ±0.7 (4)	35.9 ±1.3 (4)	36.9	34.5
Mertensia maritima				
5°	0.4 ±0.1 (2)	76.7 ±4.6 (2)	77.1	202
10°	0.3 ±0.1 (2)	73.1 ±3.5 (2)	73.4	271
15°	0.4 ±0.3 (2)	74.3 ±1.9 (2)	74.7	201
20°	0.4 ±0.2 (2)	68.7 ±2.1 (2)	69.1	186
25°	0.1 ±0.1 (2)	56.7 ±5.2 (2)	56.8	473
Crithmum maritimum				
5°	5.8 ±1.5 (4)	38.3 ±0.5 (4)	44.1	6.6
10°	9.3 ±3.0 (4)	45.8 ±4.4 (4)	55.1	4.9
15°	13.6 ±0.9 (4)	44.6 ±4.4 (4)	58.2	3.3
20°	9.0 ±0.6 (4)	33.0 ±1.4 (4)	42.0	3.7
25°	5.7 ±0.9 (4)	33.1 ±1.4 (4)	38.8	5.8

Means are calculated from individual values of Tables 6.1 to 6.6 and are expressed ± S.E. (standard error, see Campbell, 1967) with number of values used for each mean given in brackets.

Table 6.8 : Ligusticum scoticum, Mertensia maritima and Crithmum maritimum: calculated loss of carbohydrate (as glucose) from respiration data of Chapter 4; expressed as % loss of original (total) dry weight. The time for loss of 50% of dry weight was calculated directly from these values.

	1 day		1 week		Time to lose 50% of dry weight	
	5°	25°	5°	25°	5°	25°
Ligusticum	0.7	3.0	5.0	20.9	70	17
Mertensia	1.0	3.5	7.0	24.3	50	14
Crithmum	0.4	1.3	2.7	9.4	131	37

4. Sugar characterisation and determination.

This determination by gas liquid chromatography was only carried out on freeze-dried root material of Ligusticum scoticum due to lack of other material. The sugars found and the quantities of each are set out in Table 6.9 for each pretreated sample and for fresh root material. The chromatographic peaks for arabinose and ribose and for maltose and trehalose were too close to be separable, and the total peak area for the two components was combined in each case.

Sucrose is by far the predominant sugar found, with quantities ranging from 53 $mg.g^{-1}$ dry wt. (average) in fresh material, to 93 $mg.g^{-1}$ in material pretreated at 10°C for 2 weeks. All other sugars were at very low levels, less than 10 $mg.g^{-1}$, for most pretreatments except for the 25°C treatments, where, after 1 week the level of α and β glucose rises to 13 $mg.g^{-1}$ and after 2 weeks α and β glucose is 14 $mg.g^{-1}$ and fructose 11 $mg.g^{-1}$.

The highest total amount of sugar found in the roots by this method was 108 $mg.g^{-1}$ dry wt. (10.8%) after pretreatment at 25°C for 1 week. The same dry root sample had a soluble sugar content of 39% when measured by the Anthrone method, (see 2, above) and though each method gives consistent levels of sugars, the discrepancy between methods is rather large. However, several things must be taken into account here, including the different extraction methods and the greater number of 'steps' in the G.L.C. procedure, both of which may have contributed to the observed variation. The difference between the alcohol extraction for G.L.C. and the water extraction for anthrone sugars may also have exaggerated this discrepancy.

Table 6.9 : Individual sugars in roots of Ligusticum scoticum determined by G.L.C. analysis. All values expressed as $mg.g^{-1}$ relative to dry weight.

Fresh plants

Sugar	(a)	(b)	(c)	(d)	Mean
Arabinose/ Ribose	1.0	+	0.8	0.3	0.7*
Fructose	1.8	1.8	2.7	2.7	2.2
α & β glucose	3.1	3.1	5.1	3.8	3.8
Inositol	1.2	1.2	1.2	1.1	1.2
Sucrose	57	58	48	50	53
Maltose/ Trehalose	+	3.1	5.2	5.0	4.4*
Raffinose	2.8	3.6	+	2.6	3.0*
Total Sugars	66.9	69.8	63.0	65.5	68.3

Pretreated Plants

Sugar	10°/6d	15°/6d	20°/7d	25°/7d	10°/15d	15°/15d	20°/16d	25°/16d
Arabinose/ Ribose	+	+	1.1	0.8	0.3	1.7	1.0	1.0
Fructose	1.5	1.6	3.2	1.3	1.3	4.4	1.9	11.5
α & β glucose	2.7	2.9	4.2	13.2	2.4	5.3	2.9	14.2
Inositol	1.1	1.1	1.1	1.2	1.1	1.1	1.2	1.1
Sucrose	65	71	80	88	93	72	78	70
Maltose/ Trehalose	-	-	-	-	-	-	-	-
Raffinose	3.0	3.6	3.4	3.2	3.0	3.5	2.3	2.4
Total sugars	73.3	80.2	93.0	107.7	101.1	88.0	87.3	100.2

* means calculated on 3 values only.

\+ Sugar present but not in measurable quantities.

\- Sugar not detected.

DISCUSSION AND CONCLUSIONS

The differences found between the carbohydrate status of the three species investigated, Ligusticum scoticum, Mertensia maritima and Crithmum maritimum, are most marked in the starch concentration of the dried root material. The very low levels in Mertensia and low levels in Ligusticum are in contrast with the higher ones in Crithmum over the whole range of pretreatment temperatures used. Starch formation from soluble sugars by temperature displacement of the equilibrium (James, 1953) is favoured at higher temperatures and the consistently greater levels of starch in the southern Crithmum, irrespective of pretreatment, may reflect a predisposition for starch storage in this plant which is not shown by the northern species. When pretreatment is taken into account, Ligusticum and Crithmum show maximum starch contents after 10° and 15°C respectively. At temperatures above this, especially after longer times, the reduction in starch (to a very low level) probably represents a shift in the equilibrium, due to respiration use of sugars, which show much smaller relative decreases.

The total soluble sugars in Ligusticum and Crithmum are at very similar concentrations when considered relative to dry weight, while those of Mertensia are at a higher level. Greatest decrease of sugars with increasing temperature of pretreatment is found in Mertensia, the species which has least starch and thus least potential for maintaining sugar levels in the absence of any photosynthetic assimilation. Levels of sugars in the other two species may be almost maintained at the expense of starch, especially at higher temperatures when respiratory losses are greatest.

The higher values of the ratio for soluble sugar : starch in the northern species is in contrast with the lower values for Crithmum. This apparent predisposition to relatively more sugar may reflect the northern status of the plants by its connection with the lower temperature displacement of the starch sugar equilibrium.

The total carbohydrate (resulting from addition of mean values for starch and sugars at each pretreatment temperature) shows a decrease with increasing temperature of pretreatment, for both the northern species. The southern Crithmum has increasing levels of total carbohydrate up to 15°C

and a subsequent decrease, with increasing temperatures of pretreatment. While the changes in the northern plants may be explained by simple respiratory loss, those of Crithmum do not appear to be so straightforward. One possible explanation of the rise in total carbohydrate content between 5° and 15°C pretreatments in Crithmum is that some further storage substance, not detected by the analyses used, is being converted to detectable sugars over this range of temperature.

The total sugar for Ligusticum calculated from the sum of the individually determined sugars by G.L.C. analysis is at a much lower concentration than that found by the standard anthrone determination of total soluble sugars on the same plant material. The possible reasons for this are discussed earlier in the chapter, but this difference does not invalidate comparisons of relative changes measured by both methods. The sugar present in by far the largest concentrations in Ligusticum roots is found to be sucrose. The most striking change in sugar composition occurs after storage for 1 and 2 weeks at 25°C; after 1 week the glucose level rises and after 2 weeks both glucose and fructose levels rise. These changes at 25°C may possibly be explained as successive breakdown of starch after 1 week and as breakdown of sucrose after 2 weeks when starch reserves have been severely depleted. Other sugars present remain in reasonably constant concentrations for the range of pretreatments investigated.

Of the three species investigated in this chapter none can be said to show any temperature effect on carbohydrate status which could play a direct part in the regulation of its distribution, though all three do show some features of carbohydrate metabolism which could be related to their northern or southern distributions. It is, however, difficult to decide whether the above considerations are a cause or an effect of the plants' distributions.

CHAPTER 7

SUMMARY AND CONCLUSIONS

The purpose of this investigation has been to assess whether the temperatures apparently limiting the distribution of some British coastal species were in fact acting as a direct limitation on the plants in their natural habitats or whether other factors were involved. The reasonable continuity of the coast as a habitat and its relatively equable climate were both factors which contributed to the choice of coastal plants for this project. A study of those coastal species which showed either northern or southern distributions in Britain revealed good correlations between temperature isotherms and distribution for many species. For example, most of the species with a northern distribution in Britain show a southern limit which is apparently directly correlated with summer (July) mean temperature isotherms, while those species with southern distributions may have a summer, winter or combined temperature limitation at their northern limit. Extra weight was given to a direct temperature limitation by the way in which changes of distribution over 30 year periods could also be correlated with changes in the position of the relevant temperature isotherms over the same periods. These observations gave no indication of the stage in the life cycle at which any temperature limitation might act, and prompted the investigation of the effect of temperature on the selected plants at different stages in their life cycles in order to determine the mechanism of the limitation.

A study of seed germination at constant temperature showed that the northern Ligusticum scoticum and Mertensia maritima both gave maximum percentages in minimum times at the higher temperatures used, though some germination also occurred at lower temperatures, down to 5°C, if the experiments continued for long enough. The southern Crithmum maritimum and Glaucium flavum both showed maximum germination at the lower

temperatures used, with very little germination, or none at all, at 20°C and above. Alternation of temperatures enhanced the rate of germination of Ligusticum and Crithmum, the only species studied in this way, and most especially when the temperatures were in or close to the range at which the seeds had previously been shown to germinate well at constant temperatures. Though these temperature characteristics of germination fit well with those found previously for various northern and southern species, the range of temperatures at which germination can occur for those species studied here is such that, if dispersal were not limiting, seed could germinate anywhere in the British Isles at some time of the year. However, once germinated the young plants would not necessarily be able to withstand the prevailing temperature conditions in all parts of Britain. This suggests that the seed germination probably does not directly affect the distribution of any of the plants, rather that the distribution and habitat affects the germination which appears to be well adapted to maintaining an established colony of plants.

The other main investigation into the effect of temperature on distribution was carried out on mature plants in their winter state. The response to temperature of overall respiration rate (as oxygen uptake) at a range of experimental temperatures was used as a measure of the way in which the whole plant alters its physiology after various temperature and time pretreatments. Any temperature induced changes in response may be of great importance to the survival and thus distribution of a species under a particular climate regime, and this will be of greatest importance at the limits of distribution where the greatest stresses on the plants' physiology would be expected to occur.

Of the four species studied in detail, the two northern species Ligusticum scoticum and Mertensia maritima had respiration rates which

were approximately twice as great as those for the southern Crithmum maritimum and Limonium binervosum at any given temperature over the range of 5° to 30°C studied. These high rates of the northern species are of great ecological importance to the plants as they permit rapid growth and development during the short northern growing season. This is particularly important since the plants' survival may depend not only on successful seed production by the end of the season, but also on the accumulation of sufficient reserves of carbohydrate in the roots or other storage organs to enable them to survive the long northern winter. However, the intrinsically high respiration rates may also be ecologically limiting on the northern plants, most especially when they are subjected to abnormally high temperatures in either summer or winter. High winter temperatures will be most damaging to the northern plants when they have no leaves for photosynthetic replenishment of carbohydrate and other reserves which are continuously lost by respiration. At these high temperatures the finite reserves will be used more quickly and the plant will be more likely to exhaust these reserves and die, especially when the intrinsic respiration rate is high. Even a one degree rise in temperature may raise the respiration rate by as much as one percent, and reduce the time taken to use up all carbohydrate by a similar proportion.

The lower intrinsic respiration rates of the southern species will not result in such rapid growth as the northern plants in spring and early summer when the temperature rises, but this is not a disadvantage for these plants since their growing season is not restricted in the way it is for the northern plants. In this case the lower respiration rates may be ecologically advantageous since the plants are subjected to relatively high temperatures for long periods in the summer in their natural habitats. However, the southern plants all retain at least some leaves throughout the year and are thus able to replenish their

supply of carbohydrate under suitable light conditions even in winter. If this is so, then it seems unlikely that the suggested winter (January) temperature limitation on Crithmum acts as a result of the direct effect of temperature on the respiration rate of the mature plant, rather that limitation is by frost damage, for example, or that it acts at a different stage in the life cycle.

The rate of photosynthetic assimilation of carbohydrate, and the way in which this responds to temperature changes, is directly related to the survival of the plant and is thus of great importance, though it was not possible to measure it in this study. As already mentioned, photosynthesis may have a direct effect on the southern plants in winter, but the winter survival of all the plants may depend on the temperature dependence of both respiration and photosynthesis and the balance between these in the weeks or months immediately preceding the onset of winter.

When the respiration data of the species under investigation were considered in greater detail as Arrhenius plots, then further information relevant to their survival was obtained. Both the northern plants showed a break in gradient at experimental temperatures which appeared to correlate well with those indicated as limiting by the distribution maps. Ligusticum has breaks between 15° and 20°C, very close to the 15°C July temperature limitation of the distribution maps. However, the experiments were carried out on plants emerging from their winter dormant state and they showed little or no change in the position of the break after different times and temperatures of pretreatment, which suggests that the 15° to 20°C temperature related response is in operation at all times and does not depend on the previous treatment of the plant. The 15°C July mean temperature is that for the warmest summer month and might thus be expected to be limiting on the plant on the basis of the above discussion.

Though probably representing a critical change in the limiting enzyme reaction of respiration, as suggested by the change in the value of energy of activation, the break itself is possibly not directly related to the limitation on the plant, rather that the high actual respiration rate and subsequent loss of carbohydrate at temperatures above the break may bring about the eventual death of a plant. Death is not inevitable however, in fact the plant appears to compensate for the higher temperatures over short periods by the effective reduction of carbohydrate loss over that which would have been lost had there been no break in gradient: savings of up to 50% have been calculated at an experimental temperature of 30°C for Ligusticum which shows the importance of this kind of saving even in the short term.

Though the other northern species, Mertensia, shows a similar break in gradient of the Arrhenius plots, its responses to temperature seem to differ from those of Ligusticum in several respects. Firstly, the position of the break is usually between 20° and 25°C which is higher than the limiting isotherm of the distribution (15.5°C) but is still reasonably close when the possible effects of the usual shingle habitat and the slightly less reliable plant material are taken into account. Secondly, the break in gradient does not result in such a sharp change in energy of activation as that for Ligusticum, and, unlike Ligusticum, Mertensia does appear to show some dependence on pretreatment temperature as indicated by the way in which the energy of activation for the overall experimental temperature range falls with increasing temperature of pretreatment. While the results are less clear than for Ligusticum, it appears that the temperature limitation on the distribution of Mertensia might be brought about by a different mechanism in view of the suggested double response of activation energy to temperature even though the two effects must be interconnected and were also possibly the cause of some

of the variability of results. Both the break in gradient and the reduction in overall gradient with increasing pretreatment temperature will tend to reduce the loss of carbohydrate at higher experimental temperatures by the respiration rate being lower than would be expected, leaving the maximum response to increasing temperature after $5^{\circ}C$ pretreatments when the plant in effect emerges from its winter state and grows as fast as possible to take maximum advantage of the short northern growing season.

Though the Arrhenius plots of the southern species also show some temperature dependent responses of respiration these are less easy to correlate with survival and thus distribution of the species, and especially since there is less confirmatory evidence than for the northern species that the breaks are real effects. The low temperature range break in the Arrhenius plots of Crithmum respiration data occurs at around $7^{\circ}C$ which is reasonably close to the $4.4^{\circ}C$ January isotherm which bounds the northern limits of the British distribution. If, as suggested, this break is due to a denaturation or inactivation of one of the respiratory enzymes at the lower temperatures, then frost intolerance or just cold intolerance may be the explanation for the constraint on the distribution of Crithmum at its northern limits in Britain. Thus the actual respiration rate and loss of carbohydrate seem to play only a secondary role in the limitation of the plant, as suggested earlier on the respiratory evidence alone.

The case of Limonium is less straightforward since the Arrhenius plots show northern type breaks after low pretreatment temperatures and Crithmum type breaks after high ones. However, if more than one respiratory enzyme is involved then the breaks can be explained in the same way as for the above cases; when the plant has been accustomed to colder conditions it shows a warmth intolerant type break and likewise

when accustomed to warmer conditions it shows the cold intolerant type break, though the latter is at temperatures higher than for Crithmum so the mechanism may be different. This dual type of limitation fits well with the restricted distributional range of this plant along the Atlantic coasts of Europe and is the species, of those studied, which might be most expected to show this kind of limitation. Once again, though a knowledge of the photosynthetic response to temperature would complete the information relevant to survival and distribution, the ability to replenish carbohydrate even in the winter months means that this will be less likely to be limiting.

The single Arrhenius plot for Glaucium flavum was a straight line and as such yields no direct information on any possible temperature limitation on distribution in a form comparable to that for the other species studied. However, taking the unsatisfactory nature of a single observation into account, this species has the most varied geographical range of all the southern species studied, and may even extend to inland habitats where greater extremes of temperature may be encountered. If this is the case then the experimental range of this study may not have been great enough to show up any possible limitation, and once again there may have been a direct relationship between pretreatment temperature and response to experimental temperature, though extensive further investigation would be required before any firm conclusions could be drawn about this potentially very interesting species.

For all the plants investigated the values of energy of activation were similar over the experimental temperature ranges which corresponded most closely to those normally encountered by each plant in its natural habitat and normal distributional range. At temperatures above and below those of the natural range the energy of activation and rate of change of respiration was lower, where any change was found. Though the similarity

in E_a helps to confirm that the same reaction was being considered for each plant, the difference in form of the breaks throws some doubt on this. Further experiments with an even greater experimental temperature range might help to confirm (or refute) the suggestion if breaks of both kinds were found at the ends of single Arrhenius plots over an extended range.

The final investigation was into the carbohydrate status of the roots of mature plants after various time and temperature pretreatments and yielded results which added confirmatory evidence relevant to the survival of the plants. Both northern species had higher proportions of soluble sugars relative to starch than the southern Crithmum, after each of the various pretreatments and this reflects the northern and southern nature of the plants since starch is usually a higher temperature storage substance than soluble sugars. Conversely, the higher relative proportions of soluble sugars in the northern species ensure available substrate for respiration in these species with such high intrinsic rates, in addition to any cold resistance resulting from the sugar content of the roots. However, there is no clear temperature dependent effect apparent for any of the plants which might play a direct part in the limitation of distribution. The measured changes in carbohydrate content are of the same order as those calculated from the respiration data at the various temperatures with some variation attributable to the temperature related displacement of the starch soluble sugar equilibrium.

The extent of the evidence for temperature limitations on distribution obtained in this study can be most easily summarised by considering separately each of the five species studied in some detail.

The respiration results for the northern Ligusticum scoticum, both as actual rates and Arrhenius plots, give the most convincing experimental evidence for a direct temperature limitation on distribution as suggested

by the apparently limiting 59°F(15.0°C) July mean isotherm. The fact that the observed break in gradient occurs consistently between 15° and 20°C and independently of pretreatment conditions helps to confirm this, as does the preference of the plants for sites with a northerly aspect near the southern limits of its range. Though the temperature responses of seed germination are characteristic of a northern species and are certainly suited to maintaining any established colonies of the plant, it is unlikely that this alone would have any direct bearing on the overall distribution of the plant.

The other northern species, Mertensia maritima, shows similar but less consistent responses to temperature than does Ligusticum in both plant respiration and seed germination. There is reasonably good correlation between the apparently limiting July mean isotherm of 60°F (15.5°C) and the temperatures at which breaks in Arrhenius plots occur, between 20° and 25°C. The discrepancy between the temperatures may be partly attributable to the observed partial dependence on pretreatment temperature of the overall Arrhenius plot gradient and resulting E_a. The relationship of soil temperature to air temperature may also play a part here where plants exposed to the sun will experience temperatures much higher than those of the air when in their characteristic shingle habitat.

Of the southern species, Crithmum maritimum has been the most thoroughly investigated. The respiration experiments yielded Arrhenius plots with a break around 7°C which corresponds reasonably well with the apparently limiting January mean isotherm of 40°F(4.4°C) and this coupled with information on frost intolerance lends weight to the explanation of low temperature inactivation (by whatever means) of a respiratory enzyme which eventually restricts the plants' distribution by affecting its survival. As in the case of the northern species, the temperature

requirements for seed germination seem well suited to maintaining the species in its present distribution and as such will probably play only a subsidiary role in the determination of that distribution.

Limonium binervosum appears, on the limited evidence available, to have a more complex pattern of respiration response to temperature which is dependent on the temperature of pretreatment to which the plant has been subjected. The results are in keeping with the apparent dual temperature limitation at the northern limit in Britain and also with the limited overall distribution, and may reflect an inability to react successfully to very rapid temperature changes. This species was less thoroughly investigated than the others and further confirmatory work is required before any firmer conclusions can be drawn on the exact part played by temperature responses in the limitation of distribution.

The data on Glaucium flavum were so limited that no explanations for its distribution can be made with any degree of confidence on the basis of this work. The single experimental determination of respiration rate found this to be more in keeping with those of the northern species and not to those of the southern species, the group to which it belongs on the basis of its distribution. The seed germination characteristics were in keeping with its status as a southern species, though the whole plant needs further investigation.

A study of the temperature responses of seedlings of these five species would probably yield further information of value to the present problem. The seedlings might be expected to be more susceptible to any temperature stress to which they were subjected, especially before they have had time to build up any stored reserves of carbohydrate which may have a buffering action in mature plants.

The part played by photosynthetic assimilation of carbon will be significant to the survival of the plants and thus the response of photosynthesis to temperature would yield invaluable complementary data.

The southern species retain some leaves in winter and photosynthesis will be of importance throughout the winter in maintaining the carbohydrate balance. However, for the northern species which die back in winter, the speed of emergence of the new leaves in spring and the time taken for them to begin effective photosynthesis may make the difference between survival and death, especially after a long winter when replenishment of carbohydrate may become vitally important.

While these further investigations would clarify the total effect of temperature on the life and survival of the plants relative to their distributions, the present study has shown a connection between the limits of distribution and temperature responses of respiration, with certainty for Ligusticum scoticum and Mertensia maritima and with less confidence for Crithmum maritimum. Limonium binervosum shows a possible link between temperature and distribution, but both this and Glaucium flavum need further study.

APPENDIX 1

CONVERSION TABLE
DEGREES FAHRENHEIT TO DEGREES CENTIGRADE

^{o}F	^{o}C	^{o}F	^{o}C	^{o}F	^{o}C
32	0	48	8.9	64	17.8
33	0.5	49	9.4	65	18.3
34	1.1	50	10.0	66	18.9
35	1.7	51	10.5	67	19.4
36	2.2	52	11.1	68	20.0
37	2.8	53	11.7	69	20.5
38	3.3	54	12.2	70	21.1
39	3.9	55	12.8	71	21.7
40	4.4	56	13.3	72	22.2
41	5.0	57	13.9	73	22.8
42	5.5	58	14.4	74	23.3
43	6.1	59	15.0	75	23.9
44	6.7	60	15.5	76	24.4
45	7.2	61	16.1	77	25.0
46	7.8	62	16.7	78	25.5
47	8.3	63	17.2	79	26.1
				80	26.7

REFERENCES

Allen, S.E.(ed.)(1974): Chemical Analysis of Ecological Materials. Blackwell.

Anderson, J.E. & McNaughton, S.J. (1973): Ecology, 54, 1220 - 1233. Effects of low soil temperature on transpiration, photosynthesis, leaf relative water content and growth among elevationally diverse plant populations.

Arber, A.(1934): The Graminae: A Study of Cereal, Bamboo, and Grass. Cambridge.

Billings, W.D. & Mooney, H.A.(1968): Biol.Rev. 43, 481 - 529. The ecology of arctic and alpine plants.

Böcher, T.W., Holmen, K. & Jakobsen, K.(1968): Flora of Greenland. 2nd edition (English). P.Haase & son, Copenhagen.

Bond, T.E.T.(1952): J.Ecol. 40, 217 - 227. Elymus arenarius L.. (Biological Flora).

Boorman, L.A.(1967): J.Ecol. 55, 221 - 232. Limonium vulgare Mill. and Limonium humile Mill. (Biological Flora).

Campbell, R.C.(1967): Statistics for Biologists. Cambridge University Press.

Chapman, V.J.(1950): J.Ecol. 38, 214 - 222. Halimione portulacoides (L.)Aell. (Biological Flora).

Clapham, A.R., Tutin, T.G. & Warburg, E.F.(1962): Flora of the British Isles. 2nd edition. Cambridge University Press.

Dahl, E.(1951): Oikos, 3, 22-52. On the relation between summer temperature and the distribution of alpine vascular plants in the lowlands of Fennoscandia.

Dandy, J.E.(1958): List of British Vascular Plants. London.

Ellis, W.C.(1969): J.Chromat. 41, 325 - 334. Solvents for the formation and quantitative chromatography of trimethylsilyl derivatives of monosaccharides.

Firbas, F. & Losert, H. (1949): Planta, 36, 478 - 506. Untersuchungen über die Entstehung der heutigen Waldstufen in der Sudeten.

Fitter, A.(1978): An Atlas of the Wild Flowers of Britain and Northern Europe. Collins.

Glièr, J.H. & Caruso, J.L.(1973): Cryobiology, 10, 328 - 330. Low temperature induction of starch degradation in roots of a biennial weed.

Goldsmith, F.B. (1975): J.Biogeogr. 2, 297 - 308. The sea-cliff vegetation of Shetland.

Green, D.G. & Ratzlaff, C.D.(1975): Can.J.Bot. 53(19), 2198 - 2201. An apparent relationship of soluble sugars with hardiness in winter wheat varieties.

Higgins, P.D. & Spomer, G.G.(1976): Bot.Gaz. 137, 110 - 120. Soil temperature effects on root respiration and the ecology of alpine and subalpine plants.

Hultén, E.(1958): The Amphi-Atlantic Plants. Stockholm.

Hultén, E.(1961): The Circumpolar Plants: I - Monocotyledons, Gymnospermae and Pteridophyta. Stockholm.

Hultén, E.(1970): The Circumpolar Plants: II - Dicotyledons. Stockholm.

Hultén, E.(1971): Atlas över Växternas utbrednings i Norden. (Atlas of the distribution of vascular plants in NW Europe). Stockholm.

Iversen, J.(1944): Geol. Fören. Stockh. Förh. 66, 463 - 483. Viscum, Hedera and Ilex as climatic indicators.

James, W.O.(1953): Plant Respiration. Oxford University Press.

Lamb, H.H.(1967): Geogr. J. 133, 445 - 468. Britain's changing climate.

Malloch, A.J.C.(1970): Analytical studies of cliff-top vegetation in south-west England. PhD thesis, University of Cambridge.

Mayer, A.M. & Poljakoff-Mayber, A.(1975): The Germination of Seeds. 2nd edition. Pergamon.

Meteorological Office (1952): Climatological Atlas of the British Isles. H.M.S.O. London.

Meteorological Office (1963): Averages of Temperature for Great Britain and Northern Ireland, 1931 - 1960. H.M.S.O. London.

Meusel, H., Jäger, E.& Weinert, E.(1965): Vergleichende Chorologie der Zentraleuropaischen Flora. Volume I and Atlas Volume I. Jena.

Mooney, H.A.(1963): Ecology, 44, 812 - 816. Physiological ecology of coastal, sub-alpine and alpine populations of Polygonum bistortoides.

Mooney, H.A. & Billings, W.D.(1960): Am.J.Bot. 47, 594 - 598. Annual carbohydrate cycle of alpine plants as related to growth.

Mooney, H.A. & Billings, W.D.(1961): Ecol. Monogr. 31, 1 - 29. Comparative physiological ecology of arctic and alpine populations of Oxyria digyna.

Mooney, H.A. & Billings, W.D.(1965): Ecology, 46, 750 - 751. Effects of altitude on carbohydrate content of mountain plants.

Okusanya, O.T.(1976): Experimental investigation into the ecology of maritime cliff species. PhD thesis, University of Lancaster.

Okusanya, O.T.(1977): Physiol. Plant. 41, 265 - 267. The effect of sea-water and temperature on the germination behaviour of Crithmum maritimum L. .

Perring, F.H. & Walters, S.M.(1976): Atlas of the British Flora. 2nd edition. E.P.Publishing.

Pigott, C.D.(1968): J.Ecol. 56, 597 - 612. Cirsium acaulon (L.)Scop.. (Biological Flora).

Polunin, O.(1969): Flowers of Europe. Oxford.

Popay, A.I. & Roberts, E.H.(1970a): J.Ecol. 58, 103 - 122. Factors involved in the dormancy and germination of Capsella bursa-pastoris (L.)Medic. and Senecio vulgaris L. .

Popay, A.I. & Roberts, E.H.(1970b): J.Ecol. 58, 123 - 139. Ecology of Capsella bursa-pastoris (L.)Medic. and Senecio vulgaris L. in relation to germination behaviour.

Probert, R.J. & Thompson P.A.(1976): Sci. Hort. Amsterdam, 5(2), 139 - 151. Effects of temperature and seed coat treatments of germination of sweet-pea.

Riedmüller-Scholm, H.E.(1974): Flora, 163, 230 - 250. The temperature resistance of Alaskan plants from the continental boreal zone.

Riedmüller-Scholm, H.E.(1976): Flora, 165, 361 - 368. Effect of prolonged daylight and rise in temperature on temperature resistance behaviour of Alaskan plants in short day conditions.

Ridley, H.N.(1930): The Dispersal of Plants Throughout the World. L.Reeve & Co., Ashford, Kent.

Salisbury, F.B. & Spomer, G.G.(1964): Planta, 60, 497 - 505. Leaf temperatures of alpine plants in the field.

Scott, G.A.M.(1963a): J.Ecol. 51, 733 - 742. Mertensia maritima (L.) S.F.Gray. (Biological Flora).

Scott, G.A.M.(1963b): J.Ecol. 51, 743 - 754. Glaucium flavum Cranz. (Biological Flora).

Seddon, B.(1971): Introduction to Biogeography. Duckworth.

Simon, E.W.Minchin, A., McMenamin, M.M. & Smith, J.M.(1976): New Phytol. 77, 301 - 311. The low temperature limit for seed germination.

Stewart, W.S. & Bannister, P.(1973): Flora, 162, 134 - 155. Seasonal changes in carbohydrate content of three Vaccinium species with particular reference to V.uliginosum L. and its distribution in the British Isles.

Stewart, W.S. & Bannister, P.(1974): Flora, 163, 415 - 421. Dark respiration rates in Vaccinium species in relation to altitude.

Sweeley, C.C., Bentley, R., Makita, M. & Wells, W.W.(1963): J.Am. Chem. Soc. 85, 2497 - 2507. Gas-liquid chromatography of trimethylsilyl derivatives of sugars and related substances.

Thompson, P.A.(1970): Nature Lond. 225,827 - 831. Characterisation of the germination responses to temperature of species and ecotypes.

Thompson, P.A.(1973): Ann. Bot. 37, 133 - 154. Effects of cultivation on the germination character of the Corn Cockle (Agrostemma githago L.).

Thompson, P.A.(1974a): J.Exp.Bot. 25, 156 - 163. Germination of Celery (Apium graveolens L.) in response to fluctuating temperatures.

Thompson, P.A.(1974b): J.Exp.Bot. 25, 164 - 175. Effects of fluctuating temperatures on germination.

Thompson, P.A.(1974c): Scientia Horticulturae, 2, 35 - 54. Characterisation of the germination responses to temperature of vegetable seeds: 1. Tomatoes.

ed. Tutin, T.G.(et al.)(1964): Flora Europaea, Volume 1. Lycopodiaceae to Platanaceae. Cambridge University Press.

ed. Tutin, T.G.(et al)(1968): Flora Europaea, Volume 2, Rosaceae to Umbelliferae. Cambridge University Press.

ed. Tutin, T.G.(et al)(1972): Flora Europaea, Volume 3. Diapensiaceae to Myoporaceae. Cambridge University Press.

ed Tutin, T.G.(et al)(1976): Flora Europaea, Volume 4. Plantaginaceae to Compositae (and Rubiaceae). Cambridge University Press.

Wager, H.G.(1941): New Phytol. 40, 1 - 18. On the respiration and carbon assimilation of some arctic plants as related to temperature.

Wallén, C.C.(ed.)(1970): Climates of Northern and Western Europe. World Survey of Climatology, Volume 5. Elsevier, Amsterdam.

Ward, C.M.(1976): Ann. App. Biol. 83, 149 - 155. The influence of temperature on weight loss from stored onion bulbs due to desiccation, respiration and sprouting.

Wareing, P.F. & Phillips, I.D.J.(1970): The Control of Growth and Differentiation in Plants. Pergamon Press.

Williams, B.L. & Wilson, K.(eds.)(1975): A Biologists Guide to Principles and Techniques of Practical Biochemistry. Arnold.

ADDENDA

Dixon, M. & Webb, E.C.(1964): Enzymes. 2nd edition. Longmans.

Larcher, W., Heber, U. & Santarius, K.A.(1973): Limiting temperatures for life functions; Chapter III(Plants), pp. 195 - 263 in Precht, H., Cristopherson, J., Hensel, H. & Larcher, W. (1973): Temperature and Life. Springer-Verlag.

Lyons, J.M. & Raison, J.K.(1970): Pl. Physiol. 45, 386 - 389. Oxidative activity of mitochondria isolated from plant tissues sensitive and resistant to chilling injury.

Massey, V.(1953): Biochem.J. 53, 72 - 79. Studies on fumarase, 3: the effect of temperature.